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THE UNIVERSITY OF ALBERTA

ANTIFUNGAL SESQUITERPENOIDS FROM AN ARTHROSPORAE FUNGUS

by

ETCHRI AMOUZOU

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF CHEMISTRY

EDMONTON, ALBERTA

Spring 1986

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External Examiner

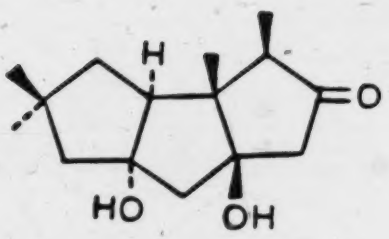
Date: 03/10/86

Cette thèse est dediée à ma famille et à mon pays, le Togo.

Abstract

A fungus observed to inhibit the growth of several tree disease causing fungi of the genus *Ceratocystis* was isolated by Drs. Y. Hiratsuka and A. Tsuneda of the Northern Forest Research Centre in Edmonton. The fungus, identified as a member of the Arthrosporae family, when grown on potato dextrose agar produces substances which inhibit the growth of *C. ulmi* (the causative agent of the so-called Dutch elm disease), *C. huntii*, and *C. montia* (two of the fungi responsible for the blue stain disease of pine).

This thesis describes the separation, isolation, and identification of the metabolites produced by the Arthrosporae fungus. The fungus produces a number of sesquiterpenoids to which structures **20 - 26** have been assigned. The structure elucidation and the stereochemical assignments for each metabolite will be discussed.



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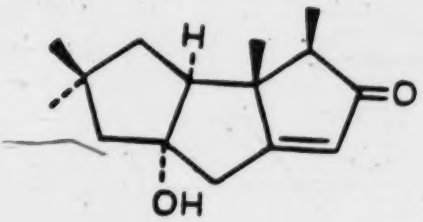

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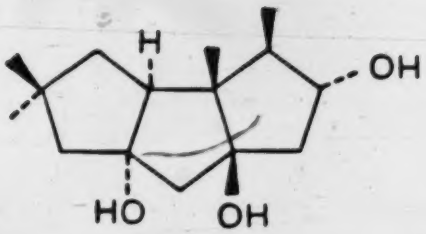

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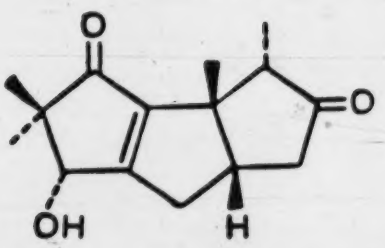

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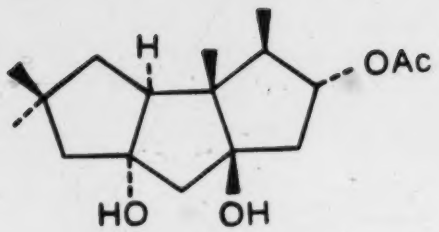

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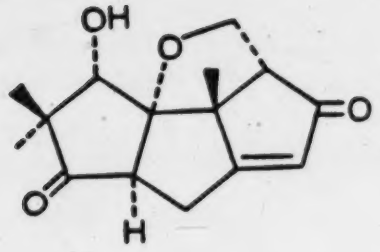

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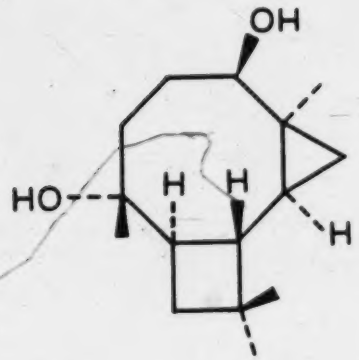

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TABLE OF CONTENTS

		Page
Abstract		v
Acknowledgements		vii
List of Tables		ix
List of Figures		x
List of Schemes		xi
I.	Introduction	1
II.	Discussion	13
	Arthrosporone (20)	14
	Anhydroarthrosporone (21)	25
	Structure and Stereochemistry of Arthrosporone (20)	45
	Arthrosporol (23)	58
	Dehydroarthrosporodione (23)	72
	4-O-Acetylarthrosporol (24)	77
	Tetracyclic ether (25)	79
	Isocyclohumuladiol (26)	82
III.	Experimental	92
	Growth of the fungus	93
	Extraction of the crude metabolites	94
	Isolation of arthrosporone (20), tetracyclic ether (25) and isocyclohumuladiol (26)	96
	Isolation of anhydroarthrosporone (21)	100
	Isolation of dehydroarthrosporodione (23) and O-acetylarthrosporol (24)	103
	Study of the Biological Activity of Arthrosporae Metabolites using a Paper Disk Diffusion Method	127
	Bibliography	133
	Appendix	141

LIST OF TABLES

Table		Page
1.	Taxonomy of Arthrosporae fungus	4
2.	Partial structures of arthrosporone (**20**)	19
3.	^{1}H NMR spectral data for anhydrarthrosporone (**21**)	36
4.	^{1}H NMR spectral data for compound **47**	38
5.	^{1}H NMR spectral data for alcohol **47**	40
6.	^{1}H NMR spectral data for alcohol **48**	42
7.	^{1}H NMR spsectral data for arthrosporone (**20**)	48
8.	^{1}H NMR spectral data for arthrosporone monoacetate (**27**)	48
9.	^{1}H NMR spectral data for arthrosporone (**20**) and *iso*arthrosporone (**57**)	52
10.	^{1}H NMR spectral data for *iso*arthrosporone	52
11.	^{1}H NMR spectral data for arthrosporone monoacetate (**27**) and diacetate (**58**)	54
12.	^{1}H NMR spectral data for arthrosporol (**22**) acetoxyalcohol **48** and diol **52**	61
13.	IR spectral data for arthrosporol acetates **66 - 68**	63
14.	IR spectral data for arthrosporol diacetate in CCl_4 solution	63
15.	^{1}H NMR spectral data for arthrosporol (**22**) and its acetates **66 - 68**	65
16.	HRMS for triols **64 - 65**	68
17.	^{1}H NMR spectral data of triols **64 - 65**	68
18.	^{1}H NMR spectral data for arthrosporol (**22**) and *iso*arthrosporol **71**	73
19.	Chemical shifts of C-11 protons in **25**, **77** and **78**	83
20.	^{1}H NMR spectral data of *iso*cyclohumuladiol (**26**)	89

LIST OF FIGURES

Figure		Page
1.	Photo of Arthrosporae fungus	5
2.	400 MHz ^{1}H NMR spectrum ($CDCl_3$) of arthrosporone (**20**)	142
3.	400 MHz ^{1}H NMR spectrum ($CDCl_3$) of anhydroarthrosporone (**21**)	143
4.	Newman projection down the C-4, C-3 bond ring C.	38
5.	Newman projection down the C-4, C-3 bond	42
6.	Ring C viewed from the top	49
7.	400 MHz ^{1}H NMR spectrum ($CDCl_3$) of arthrosporol (**22**)	144
8.	^{1}H coupling constants in the minor alcohols	70
9.	^{1}H coupling constants in the major alcohols	70
10.	Stereochemistry and coupling constants for the ring C protons in *iso*arthrosporol (**71**)	71
11.	400 MHz ^{1}H NMR spectrum ($CDCl_3$) of dehydroarthrosporodione (**23**)	145
12.	400 MHz ^{1}H NMR spectrum ($CDCl_3$) of 4-O-acetyl-arthrosporol (**24**)	146
13.	400 MHz ^{1}H NMR spectrum ($CDCl_3$-D_2O) of tetracyclic ether **25**	147
14.	400 MHz ^{1}H NMR spectrum ($CDCl_3$) of tetracyclic ether **25**	148
15.	400 MHz ^{1}H NMR spectrum ($CDCl_3$) of *iso*-cyclohumuladiol (**26**)	149

LIST OF SCHEMES

Scheme		Page
I.	In vitro transformation of hirsutic acid	9
II.	^{1}H NMR coupling pattern for arthrosporone (**20**)	17
III.		21
IV.		21
V.	^{1}H NMR coupling pattern for anhydroarthrosporone (**21**)	26
VI.	^{1}H NMR coupling pattern for O-acetylanhydroarthrosporone (**34**)	26
VII.		28
VIII.	^{1}H NMR coupling pattern for compound **41**	32
IX.		43
X.		43
XI.		51
XII.		51
XIII.		55
XIV.		57
XV.	^{1}H NMR coupling pattern for arthrosporol (**22**)	59
XVI.	^{1}H NMR coupling pattern for dehydroarthrosporodione (**23**)	76
XVII.	^{1}H NMR coupling pattern for 4-O-acetylarthrosporol (**24**)	79
XVIII.	^{1}H NMR coupling pattern for tetracyclic ether **25**	81
XIX.	^{1}H NMR coupling pattern for isocyclohumuladiol (**26**)	85

I. INTRODUCTION

An as yet unidentified Arthrosporae fungus was encountered accidentally by Tsuneda and Hiratsuka[1] at the Northern Forest Research Centre in Edmonton. The discovery, reminiscent of the *Penicillium* case,[2] was made when a culture of a pathogenic fungus, *Ceratocystis ulmi*, became contaminated with the unknown fungus. It soon became apparent that the contaminant, when grown on potato dextrose agar (PDA), produces substances which inhibit the growth of several tree disease causing fungi of the Ceratocystis family.[3,4]

C. ulmi represents the perfect stage of an Ascomycetous fungus described as *Ceratostomella ulmi* by Buisman in 1923. This fungus also reproduces by means of an imperfect stage, *Pesotum ulmi*,[5] described by Schwarz in 1922. *C. ulmi* induces vascular disease in elm trees. The disease has become known as the "Dutch elm disease", because it was a Dutch botanist who first called attention to the disease in Holland in 1919.[6] The infection was soon thereafter discovered in several parts of Europe and Asia.

The earliest known cases of the disease in North America were recorded in 1930 in Cleveland and Cincinnati. From 1930 to 1978 the disease spread over several parts of the continent.

The occurrence of Dutch elm disease in Canada was first observed in 1944 in the Province of Quebec. From 1944 to 1975, the disease gradually spread over other parts of Canada. The most recent incidence of the disease was noted in Manitoba in 1975. Newfoundland, Nova Scotia and the three most western provinces are still relatively free of the disease.* Elm trees are found in most parts of the north temperate zone and are planted in urban areas and in the countryside to provide shade, shelter and beauty.

The Dutch elm disease, called "the disease of the 20th century" is the most devastating world-wide epidemic plant disease. Since its discovery in 1919, Dutch elm disease has killed millions of elm trees in Europe and North America, causing billions of dollars in direct economic losses and inflicting inestimable damage to the aesthetic quality

---

* The infections are already recorded in California and Manitoba. There is no reason to believe that the Dutch elm disease will not spread to the three most western provinces.

of the landscape. *C. ulmi*, causal agency of the disease, invades the xylem vessel where it multiplies by yeast-like budding. The symptoms of the disease include dwarfing and wilting of the leaves, which at the same time become yellow or brown. Defoliation then takes place, followed by the death of branches of the tree. The fungal infection is spread by bark beetles, insects of the Scolytidae family. These beetles not only disseminate the spores, but also introduce them into the deeper tissues of the host plant.

Considerable progress has been made in controlling the disease.[1] Several preventive (Prophylaxis) and curative (Therapy) methods are in use, but none has yet provided satisfactory control. Extensive use of pesticides to eradicate the bark beetles and the pathogenic fungus does not seem to be the answer since this causes environmental contamination. It is imperative to find new biological tools which may be used in the future to control plant diseases. These methods should not harm the host and should not pollute the environment.

Recently, two new biological tools have found application in the control of Dutch elm disease.[4-7] One involves the manipulation of behavior of the beetles with pheromones and host attractants. Aggregation pheremones found to be attractive to the European elm bark beetle have been identified and synthesized. The active pheromone is a three-part mixture of the elm-produced cubebene (1), and the two beetle-produced metabolites α-multistriatin (2) and 4-methyl-3-heptan-ol (3).

The second new method involves the use of *Pseudomonas syringae*, a bacterium which grows on the elm sap. *P. syringae* is injected into the elm tree where it produces antimycotics toxic to *C. ulmi*. The bacterium and its antifungal metabolites are not toxic to the host. The disadvantage of this method is that every diseased tree must be treated individually. The discovery of the unidentified Arthrosporae fungus which is antagonistic to *C. ulmi* was of interest to scientists of the Canadian Forestry Service.[8] Our role has been to isolate and identify the metabolites of this interesting fungus and, if possible, to determine the compounds responsible for the antifungal activity.

The Arthrosporae fungus (UAMH* 4262) is a haploid basidiomycete.[9] Arthrosporae

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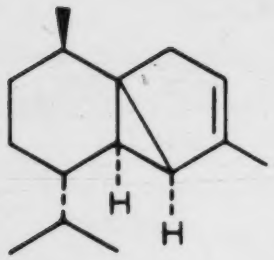
*Identification code of the Arthrosporae fungus deposited at the University of



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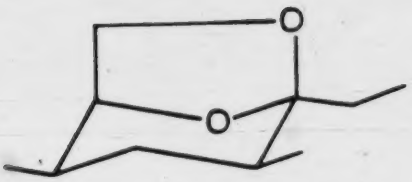

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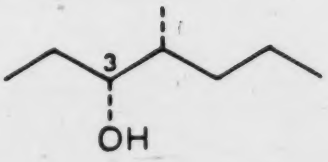

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is the form-family-genera nomenclature of a group of imperfect fungi.[10] The classification of the Arthrosporae fungus is illustrated in Table 1. Observation under a microscope shows that the mycelium of the fungus UAMH 4262 is made of several branched septate hyphae (Fig. 1). In competitive culture, the strain UAMH 4262 is highly inhibitory toward *C. ulmi* and other wood decaying fungi of genus *Ceratocystis* including the causative agents of the blue stain disease of pine.[4]

This thesis describes the efforts made towards the isolation and identification of the metabolites of strain UAMH 4262. The antifungal activity was found mainly in the neutral fraction of the broth extracts. The mycelium extracts were biologically inactive. Antibacterial activity was observed in the acidic fraction of the broth extracts.

The neutral fraction consists mainly of C_{15} secondary metabolites. The structural studies led to the conclusion that the metabolites of strain UAMH 4262 are hirsutane-like sesquiterpenoids. Sesquiterpenes are C_{15} compounds biogenetically derived from three C_5 "isoprene" units (isopentenyl pyrophosphate) which are in turn formed from acetate via mevalonate.[13] The Arthrosporae metabolites possess a tricyclo[6.3.0.$0^{2,6}$] undecane

---

*(cont'd) Alberta Mold Herbarium under the accession number UAMH 4262.

Table 1. Taxonomy* of Arthrosporae Fungus Accession No. UAMH 4262

Kingdom	Fungi
Division	Eumycotina (true or mycelial fungi)
Subdivision	Higher fungi
Class	Deuteromycotina (Imperfect fungi) (Fungi Imperfecti)
Form Sub-class	Hyphomycetidae (conidial fungi)
Form-family-genera	Arthrosporae (Section VII)
Species	Unknown
Accession No.	UAMH 4262

---

*The classification was based on information we gathered from References 10 to 14.

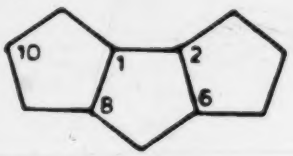
Fig. 1. Photo of Arthrosporae fungus. Courtesy of L. Sigler.*

*Curator at UAMH.

Fig. 1. Photo of Anthrosporae Fungus (X 830)
Courtesy of L. Sigler*

*Curator at UAMH

carbon skeleton (4). This linearly-fused tricyclic system (triquinane) is present in many sesquiterpenes which possess the hirsutane (5) and capnellane (6) structures.



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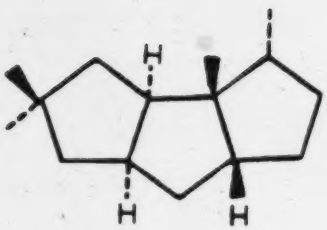

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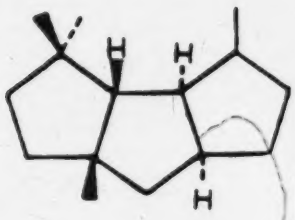

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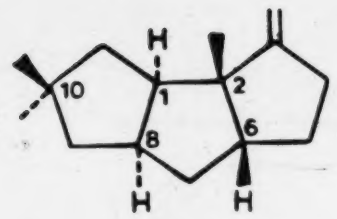
The three rings in both compounds are fused in the most thermodynamically stable *cis-anti-cis* configuration. The main difference between the two isomers is in the regiochemistry of the angular and geminal methyl groups. The various triquinane sesquiterpenes differ in the number and position of oxygen atoms in the molecule.

Hirsutene (7),[16] the parent hydrocarbon of the hirsutane-like sesquiterpenes has received much attention as a target in the synthesis of linearly fused cyclopentanoids.[17]

The most important members of the hirsutane-like sesquiterpenes are the coriolins[18] (8-11). Coriolin (8) was isolated from the culture broth of a Basidiomycetous fungus *Coriolus consors* in 1969, and its structure was elucidated two years later by Takahashi and co-workers.[19]

Diketocoriolin (10), the oxidation product of coriolin B (9) has antifungal and antimicrobial properties. Modification of the ester groups led to no loss of biological activity.[20] Kunimoto and Umezawa observed that the mode of action of diketocoriolin B on mammalian cells involved inhibition of Na-K-ATPase.[21]



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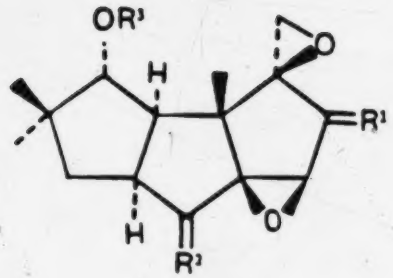


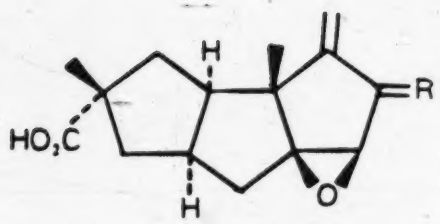
8 $R^1 = O$, $R^2 = \alpha$-OH, $R^3 = H$ **coriolin**

9 $R^1 = R^2 = \alpha$-OH, $R^3 = CO(CH_2)_4CH_3$ **coriolin B**

10 $R^1 = R^2 = O$, $R^3 = CO(CH_2)_4CH_3$ **diketocoriolin**

11 $R^1 = O$, $R^2 = \alpha$-OH, $R^3 = COCHOH(CH_2)_3CH_3$ **coriolin C**

Hirsutic acid C (12) was isolated from a Basidiomycetous fungus, *Stereum hirsutum* by Heatley *et al.* (1947)[22] and its structure was elucidated by Scott *et al.* twenty years later.[23]



12 R = **hirsutic acid C**

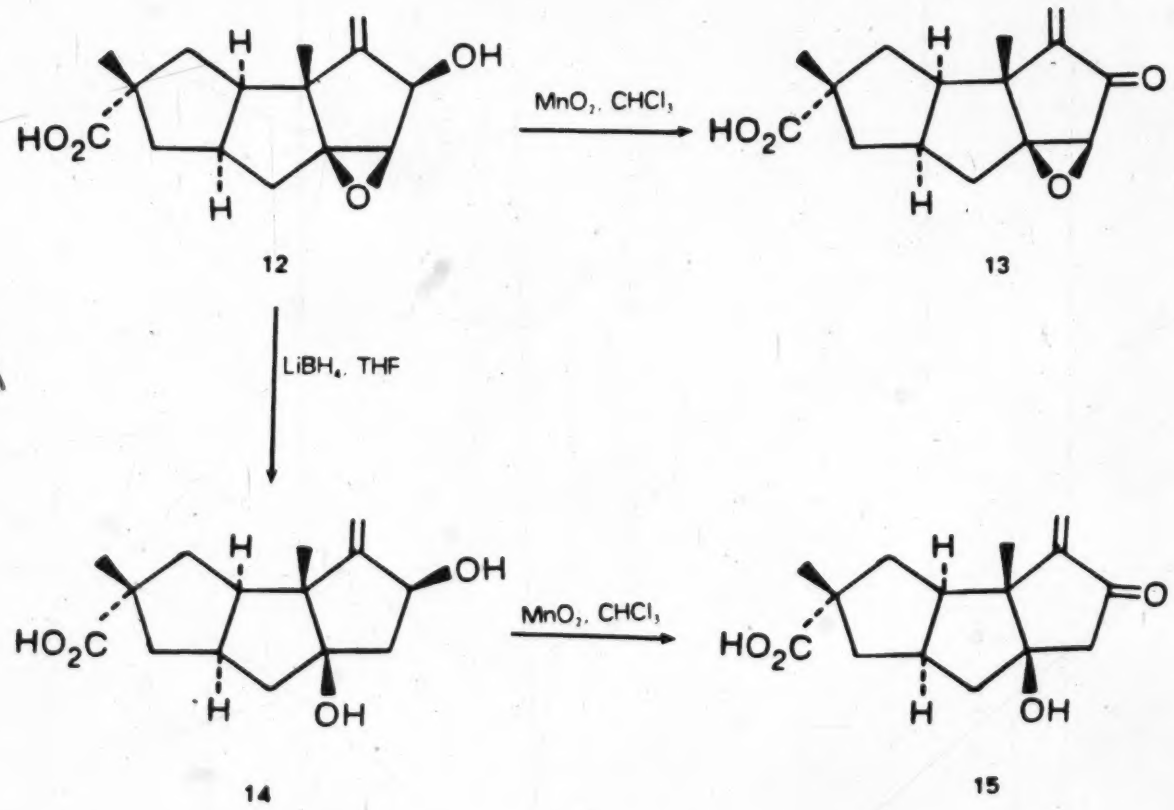
13 R = O **complicatic acid**

Mellows and Williams reported the isolation and identification of complicatic acid (**13**) from a culture of *Stereum complicatum*.[24] Hirsutic acid was also isolated from the culture as a minor metabolite. Mellows and co-workers demonstrated that by carefully choosing certain culture media and growth conditions, complicatic acid (**13**) could be converted into hirsutic acid (**12**).

Oxidation of the allylic hydroxy group of hirsutic acid using manganese dioxide gave complicatic acid (**13**). The ketone **15** obtained from hirsutic acid (**12**) *via* the diol **14** showed approximately equivalent antimicrobial properties to complicatic acid. Hirsutic acid and diol **14** showed no biological activity. Thus the epoxide does not intervene in the activity.

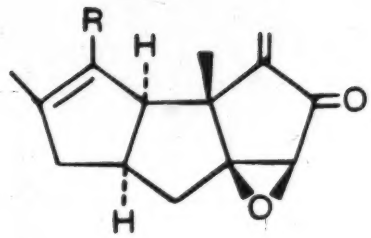
Pleurotellol (**16a**) and pleurotellic acid (**16b**) are secondary metabolites produced by a Basidiomycetous fungus *Pleurotellus hypnophilus*.[25] They possess a rearranged hirsutane structure and may be biogenetically derived from hypnophilin (**17**), a co-isolated metabolite. The three metabolites inhibit the growth of several bacteria. Furthermore, hypnophilin and pleurotellol are cytotoxic.[26]

Scheme I. In Vitro Transformation of Hirsutic Acid



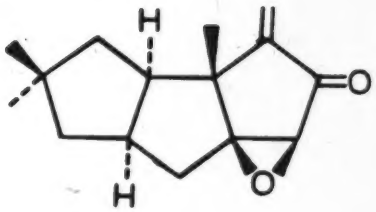
12 13

14 15



16 a R = CH_2OH

b R = CO_2H

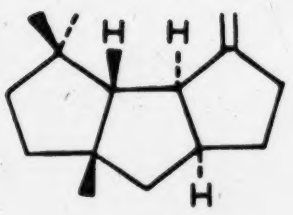


17

Capnellane-like compounds are the other known members of the linearly-fused tricyclic sesquiterpenes. Capnellene (**18**), the parent hydrocarbon of the oxygenated capnellanoids (**19**)[27] was isolated from the soft coral *Capnella imbricata* by Djerassi *et al.*[28]

It has been suggested that the capnellane-like sesquiterpenes may serve to deter attack by predators and defend against microorganisms. In the last few decades, partial or total syntheses of these linearly-fused triquinanes have posed a challenge to synthetic chemists. Several synthetic routes have been developed.[29]

The Arthrosporae fungus metabolites represent the latest members of the hirsutane-like sesquiterpenes to be identified. This thesis reports the isolation of these antifungal metabolites. They have been characterized by spectroscopic data and chemical transformations. The four major metabolites have been identified as the keto-diol **20**, the unsaturated ketone **21**, the triol **22** and the unsaturated diketone **23**. Metabolites **20, 21** and **22** possess a hydroxyl group at C-8, a characteristic feature of arthrosporonoid sesquiterpenes. C-8 is not known to be hydroxylated in the hirsutane-like sesquiterpenes. These new members of the hirsutane family have been named arthrosporone (**20**),



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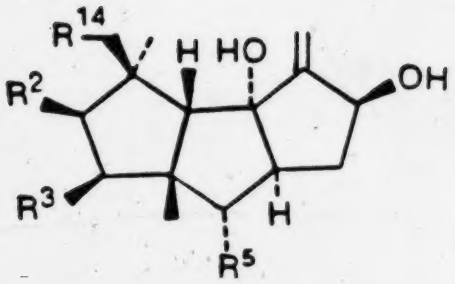


19 a $R^2 = R^3 = R^{14} = H$, $R^5 = OH$

b $R^3 = R^5 = R^{14} = H$, $R^2 = OH$

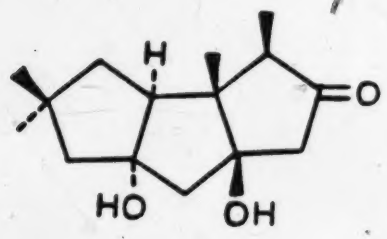
c $R^2 = R^3 = R^{14} = H$, $R^5 = OH$

d $R^2 = R^3 = H$, $R^5 = R^{14} = OH$

anhydroarthrosporone (21), arthrosporol (22) and dehydroarthrosporodione (23). Three minor metabolites were also isolated and assigned structures 24, 25 and 26. Compound 24, which has a similar R_f to that of compound 23, was identified as the monoacetate of

the triol **22**. Its structure was confirmed by selective acetylation of arthrosporol **22**.

Further research is required to confirm structures **25** and **26**.



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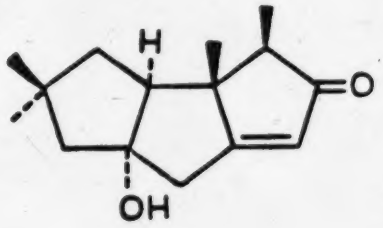

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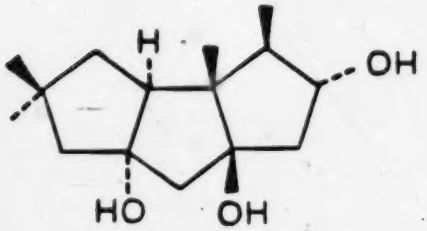
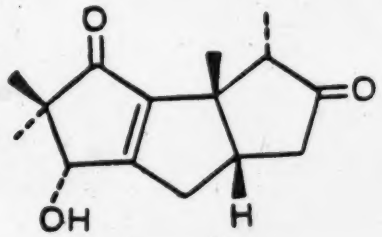


22



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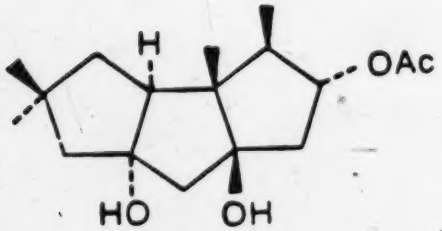

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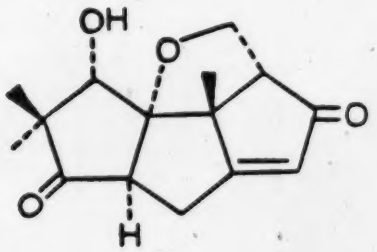

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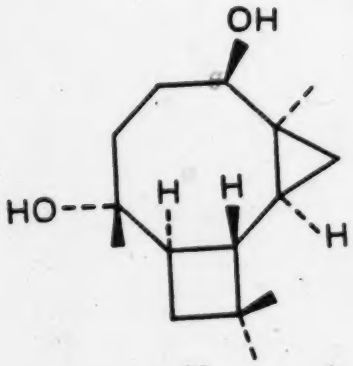

26

II. DISCUSSION

Still cultures of the Arthrosporae fungus were grown in a liquid medium composed of potato dextrose broth (PDB, 21 g/L) containing yeast extract (0.2 to 0.3%). A preliminary growth study of the Arthrosporae fungus had shown that a temperature range of 15 to 20°C is required and a 30-day incubation period is needed to obtain a good yield of broth extracts.

At the end of the fermentation period, the mycelia were separated from the broth by filtration. The broth was concentrated to one-fifth of its original volume and extracted with diethyl ether using a continuous extractor. The diethyl ether solution was washed, dried, and concentrated to give a strong smelling yellow-brown residue (≃ 1 g / 10 L).

The residue was redissolved in ethyl acetate and separated into neutral and acidic fractions by extraction with an aqueous solution of 5% NaOH. The neutral ethyl acetate solution was dried and concentrated to give ≃ 0.6 g neutral fraction. The residue was subjected to an antibiotic bioassay using the disk-diffusion method (see Experimental). The neutral extract displayed strong antifungal activity against *Candida albicans*, and several fungi of the genus *Ceratocystis* including *C. ulmi*, the pathogenic agent of the so-called "Dutch elm disease," *C. huntii* and *C. minor*, both causal agents of the so-called "blue stain disease" in pine.

Subsequently it was found that the active metabolites could be obtained as a tractable mixture simply by extraction of the broth with diethyl ether. These extracts were obtained by stirring the unconcentrated broth and ether at room temperature using a mechanical stirrer. The residue obtained after the removal of the solvent (300 to 400 mg / 10 L of fermentation broth) displayed a biological activity equivalent to that of the crude neutral broth concentrate extracts.

The sodium hydroxide extract was acidified and extracted with ethyl acetate. Concentration of the dried ethyl acetate fraction gave crude acidic material (0.250 g). The acidic extract, which also contained most of the colored compounds, displayed a relatively good antibacterial activity against *Staphylococcus aureus*. However no extensive investigation of the acidic mixture was undertaken due to its complexity and the lack of

major components as evidenced by excessive tailing of the TLC of the mixture.

The mycelia were air-dried and extracted with diethyl ether, then with ethyl acetate in a Soxhlet extractor. The mycelial extracts, obtained in larger amounts than the broth extracts, were subjected to antibiotic testing and were found to be devoid of antibacterial and antifungal activities. Separation of the mycelial extract led to the isolation of a large quantity of hydrocarbons, fatty acids, ergosterol and ergosterol peroxide (probably an artifact from ergosterol) along with several *ortho*-phthalates. These were not investigated in detail.

The biologically active neutral fraction of the broth extract was separated by column chromatography over silica gel. Further purification by chromatography over silica gel together with recrystallization, when necessary, led to the isolation of four major metabolites, three crystalline (**20-22**) and one liquid (**23**). Compounds **21** and **23** are UV active, while **20** and **22** are not. Compounds **20-23** are readily visualized by TLC when sprayed with 1% vanillin in sulfuric acid (reagent A) and each compound gives characteristic colour reactions. They are eluted from the chromatography column in the following order:

23 > 21 > 20 >> 22

The intensity of the antifungal activity of compounds **20-23** against *C. montia* can be ranked as follows:

21 > 22 > 20 > 23

Only compounds **20** and **21** show antifungal activity against *C. ulmi*.

Three minor compounds (**24-26**) eluted along with the major compounds will be described at the end of the discussion. Compound **24** eluted along with the major UV-active compound **23**, while compounds **25** and **26** eluted along with major compounds **21** and **20**, respectively.

Arthrosporone (20)

Arthrosporone is a rather polar solid material isolated from the Arthrosporae broth extract. It is easily recognized by its characteristic colour reaction on TLC: R'_f 0.54 (ethyl

acetate / pentane 1:1).* A reddish colour develops instantaneously when the freshly developed TLC plate is sprayed with 1% vanillin in aqueous sulfuric acid (reagent A) and then carefully heated on a hot plate. When the compound is very concentrated a reddish colour develops initially, then darkens and slowly turns into a permanent greyish blue coloration.

Impure arthrosporone is usually obtained as a yellowish gum. It is then subjected to two or three purifications using a combination of flash and normal chromatographic techniques (solvent systems: acetonitrile / dichloromethane 1:3, acetone / dichloromethane 15:85).

Arthrosporone is obtained as shiny crystals which are recrystallized from Skellysolve B / diethyl ether to give analytically pure compound **20**: m.p. 139-141°C., $[\alpha]_D^{23}$ = 140.8 ($CHCl_3$). Arthrosporone has a molecular formula $C_{15}H_{24}O_3$, accounting for a molecular weight of 252 as determined by a high resolution mass spectrum (HRMS). The molecular weight (mol. wt.) was confirmed by a chemical ionization (CI) mass spectrum, the peak at m/z 270 (100%, M+18) corresponding to a collision complex of arthrosporone and an ammonium ion (NH_4^+). The fragmentation pattern in the HRMS of **20** displays peaks at m/z 234 ($M^+ - H_2O$, 27%), 206 ($M^+ - H_2O$-CO), 192 (234-CH_2CO, 90%) and 125 ($C_7H_9O_2$, 100.0%)

The ultraviolet (UV) spectrum of arthrosporone (λ_{max} 280 nm) shows a band characteristic of the $n \rightarrow \pi^*$ transition of a ketone.[30] The Fourier transform infrared spectra (FTIR) of compound **20** shows strong bands at 3440 cm^{-1} (broad, OH), 1731 cm^{-1} (C=O) and a moderately intense doublet at 1380 and 1360 cm^{-1}. The doublet is characteristic of *gem*-dimethyl groups (C-H bending vibration).[31] From this spectral information it was concluded that this metabolite is a ketone (possibly a cyclopentanone)[32] and thus it was named arthrosporone (a ketonic metabolite from Arthrosporae). Compound **20** has four sites of unsaturation, at least one hydroxyl group (MS, IR) and a *geminal* dimethyl group (IR).

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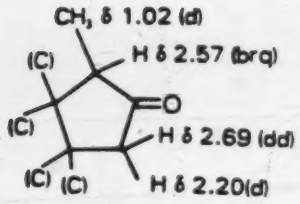
*The R'_f is the R_f using cholesterol as reference.

The ^{1}H-NMR spectrum of arthrosporone displays three methyl singlets at δ 1.16, 1.08, and 0.84, and a methyl doublet (J = 7Hz) at δ 1.02. There are no low field protons (i.e., δ > 3.0) indicating that the molecule contains no olefinic proton(s) or protons *geminal* to an oxygen atom. Therefore the hydroxyl group(s) in arthrosporone must be tertiary. The remainder of the ^{1}H-NMR (Figure 2) spectrum consists of well resolved one proton spin-multiplet systems. The lowest field proton appears at δ 2.69 as a doublet of doublets with a small coupling (J = 1 Hz) and a large coupling (J = 19 Hz). The magnitude of the large coupling is characteristic of the *geminal* coupling of methylene protons *alpha* to a five-membered ring ketone.[33] Irradiation experiments show that the *geminal* partner of the proton at δ 2.69 resonates at δ 2.20. The latter proton appears as a doublet slightly overlapping another signal (1H, d, 16Hz). Irradiation of the signal at δ 2.69 sharpens a one proton quartet at δ 2.57. This proton shows *vicinal* coupling (J = 7 Hz). These results suggest the presence of partial structure I.

The ^{1}H-NMR spectrum of arthrosporone also exhibits a group of methylene protons at δ 2.40 and 2.21, each proton appearing as a doublet (J_{gem} = 16 Hz).

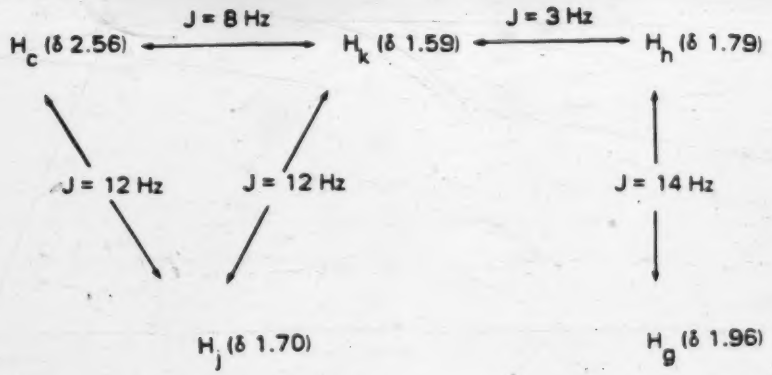
A broad quartet at δ 2.57 overlaps with a proton multiplet centered at δ 2.56. Extensive decoupling expeiments show that this multiplet is strongly coupled to two other protons, each of which show further coupling as summarized in Scheme II.

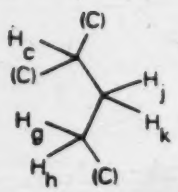
Addition of D_2O to the NMR sample simplifies the signal H_k at δ 1.59 (doublet of doublets of doublets (ddd)). The irradiation experiments show that H_k couples either through three bonds (^{3}J = 3 Hz) or four bonds (^{4}J = 3 Hz) to the proton H_h (dd) at δ 1.79. Thus the coupling is either a *vicinal* (three bonds) or a long range (four bonds) coupling. The proton at δ 1.79 shows *geminal* coupling to the proton H_g at δ 1.96 (doublet, J_{gem} = 14 Hz). When the signal H_j at δ 1.70 (triplet) is irradiated, the multiplicity of the signal H_c at δ 2.56 is strongly affected and the signal H_k at δ 1.59 (doublet of doublets) broadens. These observations suggest that protons H_j and H_k are *geminal* (J_{gem} = 12 Hz), and therefore, proton H_c is *vicinal* to H_j and H_k. Alternatively protons H_j and H_c may be *geminal* (J_{gem} = 12 Hz), and consequently, proton H_k is *vicinal*. These assumptions lead to partial structures II to IV.



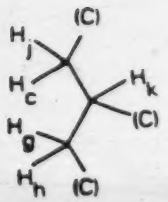
I

Scheme II. ^{1}H NMR Coupling Pattern for Arthrosporone

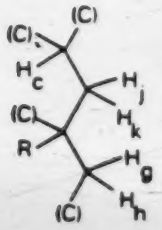




II



III



R=(C) or (O).

IV

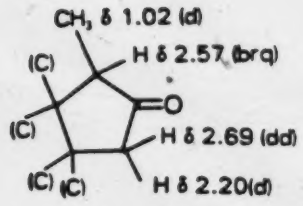
H_g appears as a doublet (J_{gem} = 14 Hz) and H_h is a doublet of doublets (J_{gem} = 14 Hz, J_{vic} = 3 Hz). The multiplicity of H_j (triplet) and H_g (doublet) suggests that arthrosporone does not have an ethylenic (-CH_2CH_2-) linkage in its structure. Therefore the part structure II is rejected. From the information gathered thus far, the structure of arthrosporone incorporates the partial structures I, III or IV, and V to VII (Table 2).

The number of hydroxyl proton(s) in compound **20** could not be obtained since these protons resonate at the same chemical shift as that of residual water in deuterochloroform. Analysis of the ^{1}H NMR spectrum of arthrosporone allows the assignment of 22 (of 24) hydrogens, which are directly attached to carbon. Therefore the structure of arthrosporone must contain two tertiary hydroxyl groups.

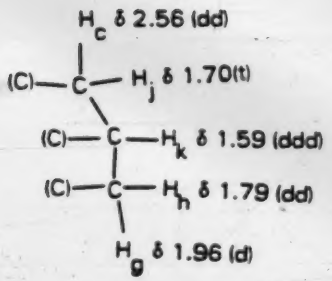
The tertiary nature of the hydroxyl groups was consistent with their resistance to oxidation reactions. However, under forcing conditions arthrosporone could be selectively converted to a monoacetyl derivative. Acetylation[34] of arthrosporone with acetic anhydride containing a trace of 4-N,N-dimethylaminopyridine in triethylamine at room temperature for three days, gave a monoacetate **27** (m.p. 120-122°C, $[\alpha]_D^{23}$ -37.6°, m/z calcd. for $C_{17}H_{26}O_4$, 294.1824, found 294.1818). The infrared spectrum of **27** shows a weak intensity broad band at 3400 cm^{-1} (OH), and strong bands at 1736 and 1243 (OAc) cm^{-1}. The ^{1}H NMR spectrum of the monoacetate **27** shows a 3-proton singlet at δ 1.98 attributed to an acetyl methyl and a D_2O exchangeable broad singlet at δ 1.47 due to a hydroxyl proton. The presence of a hydroxyl group as evidenced by the infrared and ^{1}H NMR spectra of the monoacetate **27** confirms the presence of two hydroxyl groups in the structure of arthrosporone. This information suggests that arthrosporone is a ketodiol.

The ^{13}C NMR spectrum of arthrosporone exhibits the presence of 15 carbons. The signal at δ 216.4 is the only sp^2-hybridized carbon and is assigned to the carbonyl carbon of the cyclopentanone[35] (partial structure VIII). Two signals appearing at δ 91.0(s), 87.0(s) are assigned to the two sp^3-hybridized quaternary carbons bearing the hydroxyl groups.[36] The rest of the spectrum shows the presence of two sp^3-hybridized quaternary carbons (singletx2), two methine (doubletx2), four methylene (tripletx4) and four methyl (quartet x

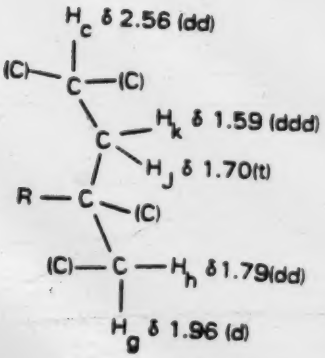
Table 2. Partial Structures of Arthrosporone



I

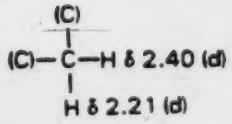


III

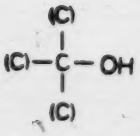


R=(C) or (O).

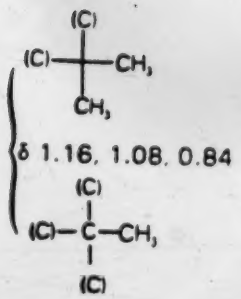
IV



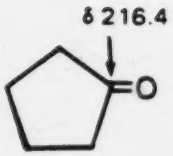
V



VI



VII

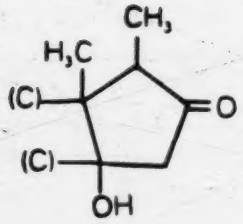


VIII

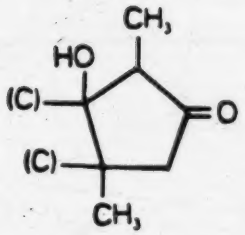
4) carbons. The number of hydrogen atoms attached to carbon in the molecule counted from the ^{13}C NMR signal multiplicity is 22. Taking into account the two hydroxyl-bound singlet carbons, a formula $C_{15}H_{24}O_3$ is obtained and this formulation agrees very well with HRMS results. The ^{13}C NMR spectrum also indicates the absence of any other sp^2-hybridized carbon,[37] thus ruling out the presence of a carbon-carbon double bond in the structure of arthrosporone. This information, in addition to the fact that the molecule has four methyl groups and four sites of unsaturation suggests that arthrosporone possesses a fused tricyclic undecanoid skeleton. Further, one of the three rings is a cyclopentanone represented by the partial structure VIII.

The base peak in the HRMS of arthrosporone, m/z 125 ($C_7H_9O_2$) corresponds to a loss of $C_8H_{15}O$. This fragment may be formed either from partial structures IX and X *via* the mechanisms proposed in Schemes III and IV. From the information derived to this point several structures may be proposed for arthrosporone (structures **28** to **33**).

The ^1H NMR spectrum of arthrosporone (**20**) was compared with the ^1H NMR spectrum of monoacetoxyarthrosporone (**27**). In **27** three protons (δ 2.83 (dd), δ 2.81 (d), and δ 2.28 (dd)) have been shifted downfield with respect to the chemical shifts observed

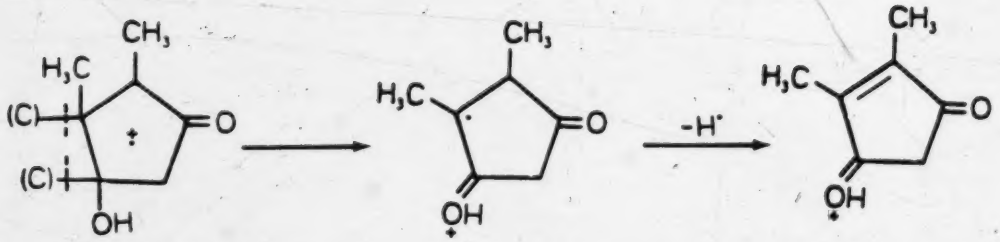


IX



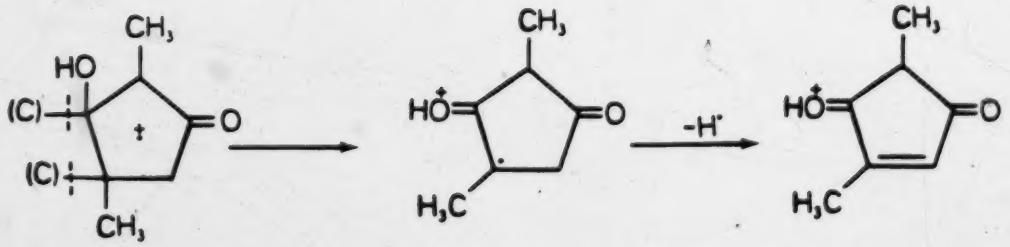
X

Scheme III



m/z 252 ($M^{+\cdot}$) m/z 126 m/z 125 (100%)

Scheme IV

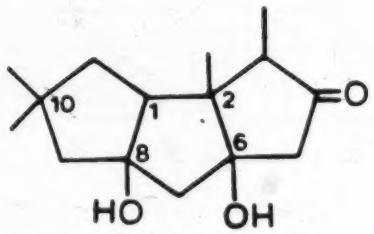


m/z 252 ($M^{+\cdot}$) m/z 126 m/z 125 (100%)

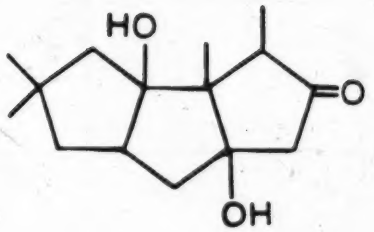
in **20** (δ 2.56 (dd), 2.40 (d) and δ 1.78 (dd)), presumably due to the anisotropic effect of the acetoxyl carbonyl.[39] In such a case a proton *vicinal* and *cis* to the hydroxyl group will be deshielded in the ^{1}H NMR when the hydroxyl group is replaced with an acetoxyl group. Of the structures tentatively proposed for arthrosporone only structures **28** to **31** are consistent with the observed acetate shift in the ^{1}H NMR (**20** → **27**). In structures **28** and **29** the *cis*-protons at C-1, C-7 and C-9 may experience a deshielding effect from the acetoxyl group, while the same results may be expected for the *cis*-protons on C-1, C-8 and C-10 in structures **30** and **31**. In structures **32** and **33**, only two *cis*-protons may experience the anisotropic effect of the acetoxyl group therefore structures **32** and **33** may be eliminated from consideration. The tentative structures proposed for arthrosporone are linearly fused triquinanes and are assumed to exist in the *cis, anti, cis* configuration as evidenced by the structures of hirsutane-like sesquiterpenes, sterpurane-like sesquiterpenes and protoilludene.[39] Since the angular methyl protons would hinder the vicinal *cis* hydroxyl group, acetylation probably would occur at C-8 (tentative structures **28** and **29**), or at C-9 (tentative structures **30** and **31**) in monoacetoxyarthrosporone **27**. In arthrosporone the methylene protons (at the carbon α to the carbonyl) *vicinal* to the 3° OH resonate at δ 2.69 and 2.20. Their chemical shifts, which remain unchanged in the monoacetoxyl derivative δ (2.64 and 2.22) are consistent with each of the proposed structures **28-31**.

The isolation of a second metabolite from the Arthrosporae broth extract and the analysis of its spectral properties made an important contribution to the structural elucidation of this class of compounds.

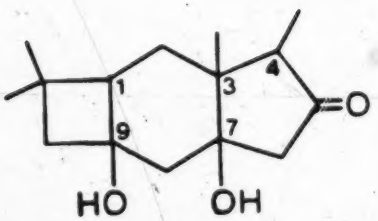
The newly isolated metabolite which is also a C_{15} compound, is UV active and its molecular formula is the same as that of arthrosporone minus one molecule of water. Indeed, arthrosporone may be converted to this metabolite by treatment with *p*-toluenesulfonic acid in benzene. We therefore named this metabolite anhydroarthrosporone. Anhydroarthrosporone (**21**) is a crystalline compound which is slightly less polar than arthrosporone (**20**). R'_f 0.74 (acetone/benzene 2:3), 0.49 (ethyl acetate/pentane 1:1, 2xdevelopment). It shows the same colour reaction as does **20** when



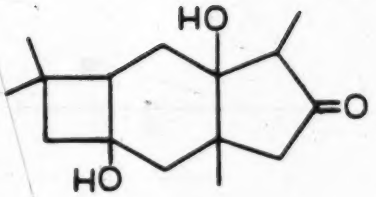
28



29



30



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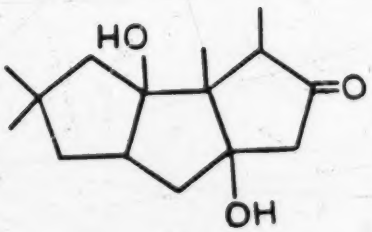

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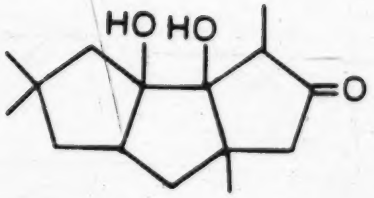


33

visualized on analytical tlc using reagent A-charring technique. Furthermore, both metabolites char green when reagent B-charring technique is used.*

Anhydroarthrosporone is an optically active compound $[\alpha]_D$ +62° ($CHCl_3$) and has a melting point of 118-119°C. Its molecular weight as determined by HRMS is 234 ($C_{15}H_{22}O_2$, M^+). The molecular weight has been confirmed by chemical ionization (CI) mass spectrum, the highest peak at m/z 235 (100%) being due to the presence of the molecular ion plus a proton (M^+H^+). The molecular formula $C_{15}H_{22}O_2$ accounts for five sites of unsaturation. Furthermore the HRMS exhibits fragment ions at m/z 216 ($C_{15}H_{20}O$, $M^+ - H_2O$) and 123 ($C_8H_{11}O$, $M^+ - C_7H_{11}O$), with a base peak at m/z 122 ($C_8H_{10}O$).

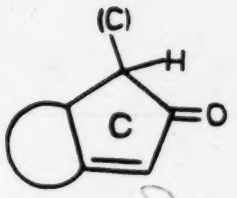
Anhydroarthrosporone is UV active as evidenced by the band at λ_{max} 230 (ϵ 13700). Its infrared (IR) spectrum shows a hydroxyl absorption (3461 cm^{-1}, strong and broad), an α,β unsaturated carbonyl absorption (1693 and 1632 cm^{-1}, both strong) and suggests the presence of a *gem*-dimethyl group (1372 and 1364 cm^{-1}, medium doublet).

The ^{13}C NMR spectrum of anhydroarthrosporone displays three low-field signals of sp^2-hybridized carbons. One of the signals (δ 211.6, weak singlet) is assigned to the carbonyl carbon, the other two signals at δ 177.0 (singlet) and δ 122.9 (doublet) are assigned to disubstituted and monosubstituted olefinic carbons respectively. A carbon doublet resonates at δ 63.4 suggesting the presence of a methine carbon α to a carbonyl.[40] These chemical shifts together with the IR and UV data suggest the presence of the partial structure XI.

The ^{13}C NMR spectrum of anhydroarthrosporone also shows the presence of a quaternary carbon bearing an hydroxyl group (δ 92.7).[41] The remainder of the spectrum consists of one singlet (a quaternary carbon), two doublets (CHx2), three triplets (CH_2x3) and four quartets (CH_3x4). The multiplicity of the carbons bearing hydrogen atoms gives 21 hydrogens plus 1 hydroxyl proton. Thus, the ^{13}C NMR spectrum confirms the molecular formula $C_{15}H_{22}O_2$.

---

* A TLC plate was developed and dipped in a reagent B (5% phosphomolybdic acid in 5% aqueous sulfuric acid containing a trace of cerium (III) sulfate). The plate was dried out and carefully heated on a hot plate.



λmax (calcd) 231 nm, obs. 230 nm

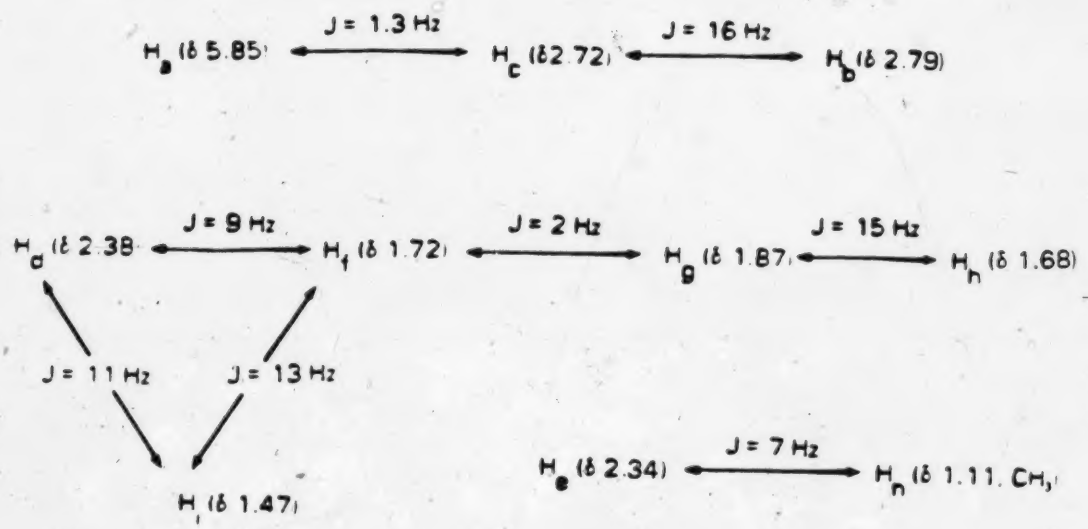
XI

The ^{1}H NMR spectrum of anhydroarthrosporone (Figure 3) displays a low field proton at δ 5.85 coupled (J = 1.3 Hz) to a proton at δ 2.72. Decoupling experiments reveal that the proton at δ 2.72 also has a *geminal* coupling (J_{gem} = 15.8 Hz) with a proton doublet at δ 2.79. (i.e., the two protons at δ 2.79 and 2.72 constitute an AB quartet centered at δ 2.76 with J_{AB} = 15.8 Hz). As well, a methyl doublet at δ 1.11 which is coupled vicinally to a one proton quartet (δ 2.34, J = 7 Hz), three methyl singlets (δ 1.22, 1.13 and 0.94) and a series of one-proton multiplets (Scheme V) are observed.

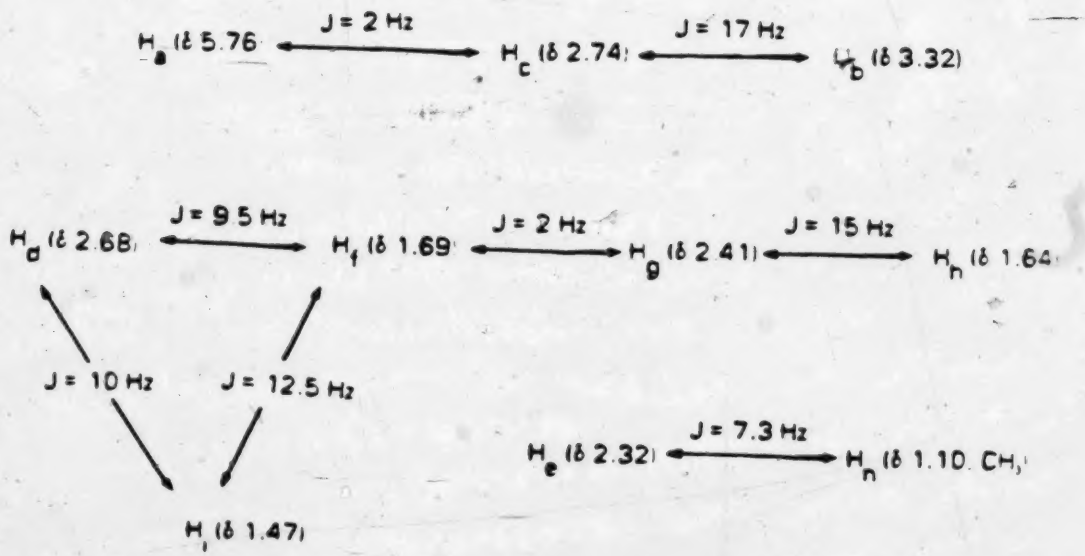
Acetylation of **21** (Ac_2O/ DMAP) in triethylamine at room temperature overnight gave O-acetylanhydroarthrosporone (**34**). $C_{17}H_{24}O_3$ (276, M^+). The IR spectrum of acetate **34** shows no OH absorption but shows strong bands at 1728 and 1241 cm^{-1} (OAc) and the α,β-unsaturated ketone at 1706 and 1630 cm^{-1}. The ^{1}H NMR spectrum of the acetate shows the presence of an acetyl methyl singlet at δ 1.98.

A detailed comparison of the ^{1}H NMR spectra of anhydroarthrosporone (**21**) and its acetate (**34**) was helpful in the determination of the structure of **21**. Extensive decoupling experiments allowed the assignment of different protons in anhydroarthrosporone (**21**) (Scheme V) and anhydroarthrosporone acetate (**34**) (Scheme VI).

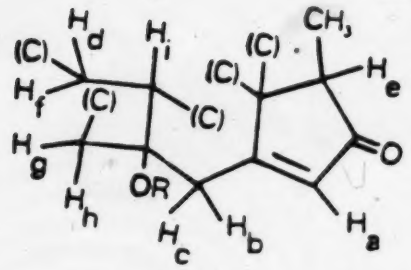
Scheme V



Scheme VI

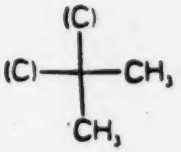


The spectroscopic data led to the formulation of partial structures XII and XIII.



XIIa R = H

XIIb R = Ac

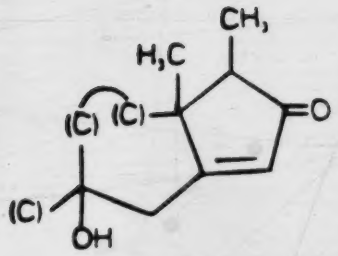


XIII

The HRMS of anhydroarthrosporone displays a base peak at m/z 122 ($C_8H_{10}O$). This peak may be accounted for *via* the proposed mechanism in Scheme VII on the assumption that the partial structure XIV is present in the molecule.

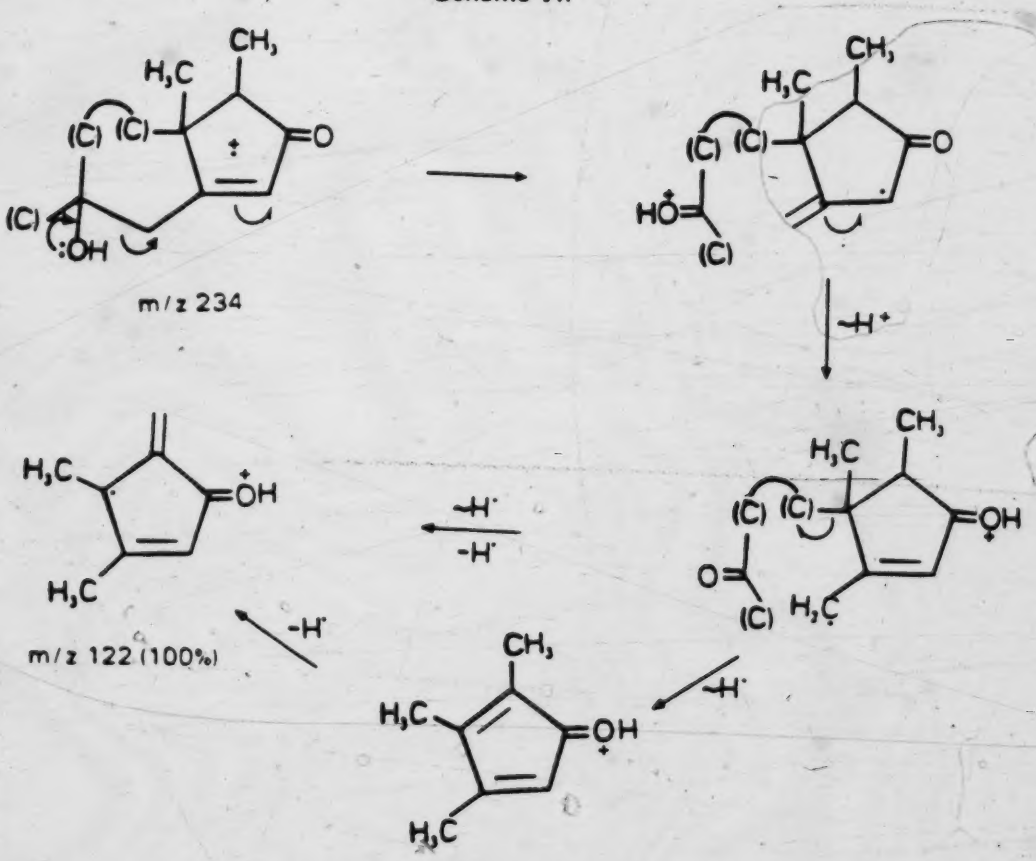
The ^{13}C NMR spectrum of anhydroarthrosporone (**21**) exhibits two quaternary carbon signals, one of which bears the *gem* dimethyl group (XIII). Since one of the substituents on the carbon β to the carbonyl in XII is a methyl group (XIV), it follows that the *gem* dimethyl group (XIII) can be incorporated in only two ways. Insertion of the *gem* dimethyl group between C-11 and C-9 followed by bond formation between C-1 and C-2 gives rise to structure **35**, whereas insertion of the *gem* dimethyl group between C-1 and C-9 followed by bond formation between C-2 and C-11 gives rise to structure **36**.

All the spectroscopic data presented for anhydroarthrosporone acetate (**34**) is consistent with either a hirsutane skeleton (**35**) or with skeleton **36**. In addition the ^1H NMR spectrum of acetate **34** shows deshielding of three protons and one of the singlet methyls with respect to the ^1H NMR spectrum of **21**. For each of structures **35** or **36** the deshielded protons would be those *vicinal* and cis to the acetoxyl group. Thus the



XIV

Scheme VII



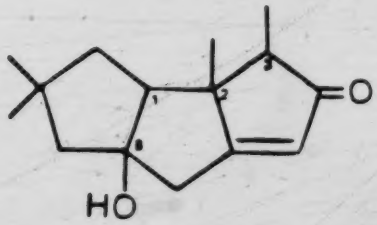
methine proton at C-1 is *cis* to the tertiary hydroxyl group at C-8.

An experiment was designed to distinguish which of the two structural types (i.e., **35** or **36**) is that of anhydroarthrosporone. Compounds such as **35** or **36** would be

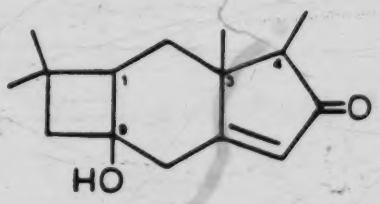
expected to undergo a retro-aldol cleavage to give bicyclic compounds **39** or **40**, respectively, *via* isomerization of the corresponding keto-dienol **37** or **38**.

Inspection of the infrared spectra of the product of the fragmentation reaction of anhydroarthrosporone will determine whether a cyclobutane or a cyclopentane moiety is present. The carbonyl of a cyclobutanone will absorb near 1780 cm^{-1}, while the carbonyl stretching band of cyclopentanone will absorb around 1745 cm^{-1}.[42]

When a mixture of anhydroarthrosporone and an excess of sodium hydride in dry benzene was allowed to stir at room temperature under nitrogen for 5 hours, a single compound (**41**) was obtained quantitatively. Compound **41** is less polar than the starting metabolite **21** and does not char with reagent A. Compound **41** is optically active ($[\alpha]_D$ -202° ($CHCl_3$)) and its UV spectrum shows a maxima at λ 223 nm, characteristic of β-alkylsubstituted cyclopentenone XV (λ_{max} (calculated) 226 nm).[43]



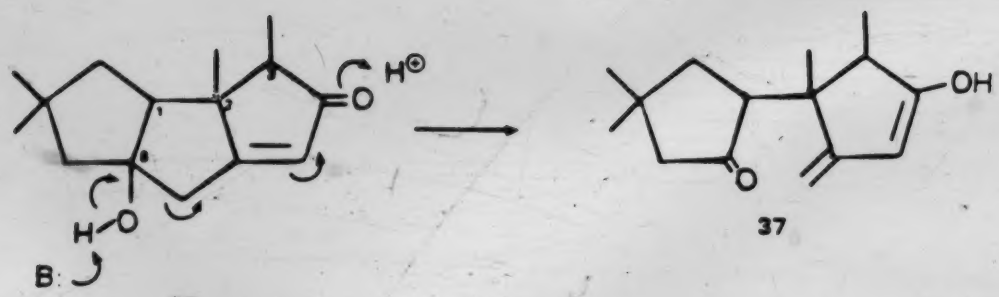
35



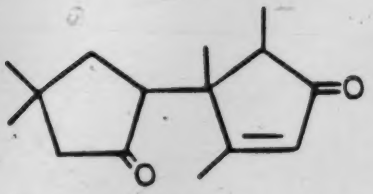
36

The IR spectrum of **41** is consistent with partial structure XV as evidenced by the strong absorption bands due to an α,β-unsaturated cyclopentenone (1702 and 1625 cm^{-1}). In addition the IR displays no hydroxyl absorption band but does show a strong carbonyl absorption band a 1738 cm^{-1}, indicative of the presence of a cyclopentanone.

Compound **41** has the same molecular formula ($C_{13}H_{12}O_3$, m/z 234, M^+) as anhydroarthrosporone **21**.



35 37



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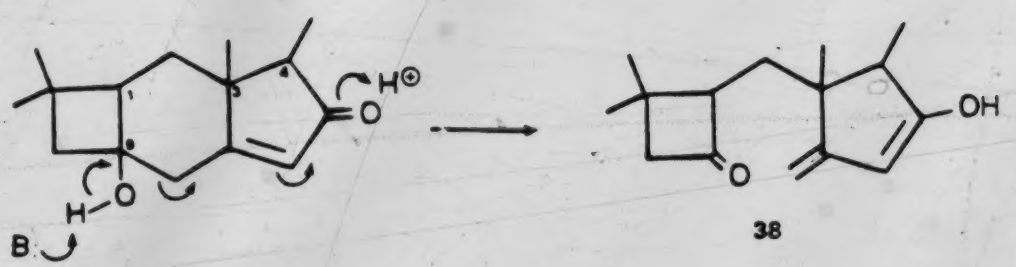
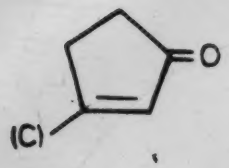
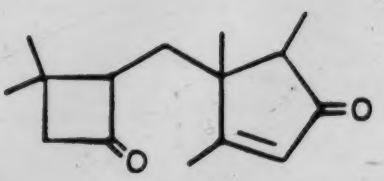


36 38

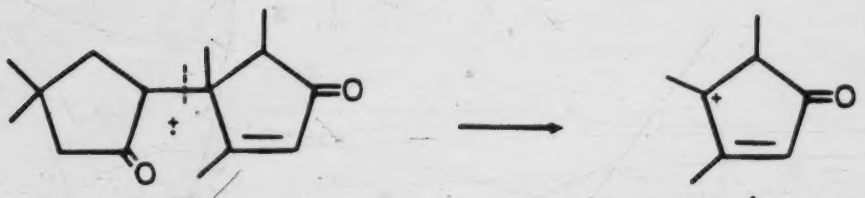


XV



40

The HRMS of **41** exhibits a base peak at m/z 123 ($C_8H_{11}O$) which can be attributed to formation of fragment A *via* the mechanism shown below



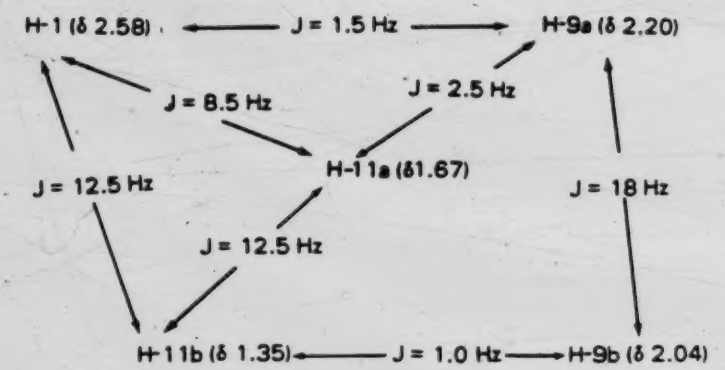
39 A

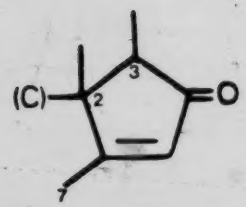
This data suggests that compound **41** has structure **39**; consequently anhydroarthrosporone (**21**) has structure **35**.

The ^1H NMR spectrum of compound **41** gives further support to the structural assignment. A low field proton (δ 5.95, q, J = 1.6 Hz) shows allylic coupling to a methyl group (δ 2.05, d, J = 1.6 Hz). Three methyl singlets (δ 1.45, 1.19 and 1.04) and a methyl doublet (δ 1.08, J = 7.5 Hz), which is coupled to a methine proton (δ 2.08, q), are observed. These data are consistent with the partial structure XVI. The remainder of the spectrum shows a set of proton multiplets (Scheme VIII).

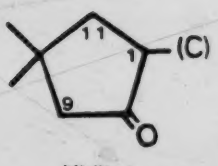
A similar spin system consistent with partial structure XVII has precedence in the literature[44] in the structure of Fomannosin (**42**) and its derivative **43**. The long range coupling (^4J = 2.5 Hz) observed in anhydroarthrosporone has also been observed in **43** and cybrodol (**44**).[45]

Scheme VIII: ^1H NMR Coupling Pattern for Compound 41

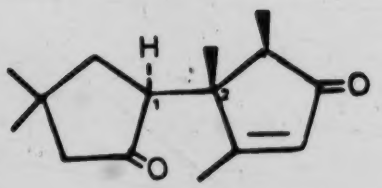




XVI

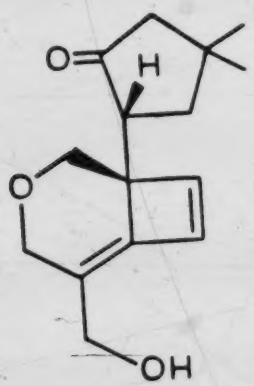


XVII



39

Bond formation between C-1 (partial structure XVII) and C-2 (partial structure XVI) leads to structure **39** for compound **41**. The stereochemistry in **39** is assigned by assuming that the stereochemistry at C-1, C-2 and C-3 is the same as the relative stereochemistry assigned to the starting anhydroarthrosporone (**21**).



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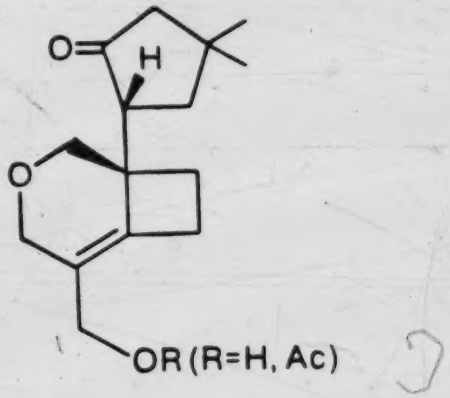
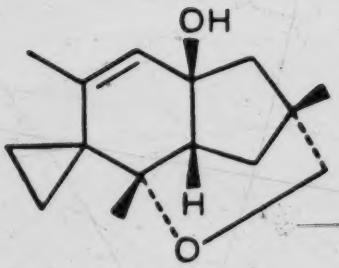
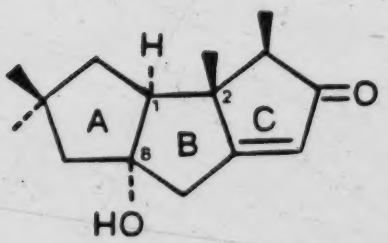


43



44



21

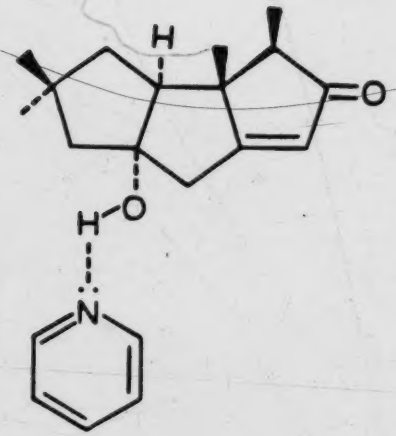
Anhydroarthrosporone possesses the hirsutane skeleton. The numbering system and ring designation are shown in structure **21**. The *cis* stereochemistry is assumed for the AB ring junction. The angular methyl at C-2 is assigned a β-orientation to satisfy the *cis, anti, cis* configuration consistently found in the hirsutane series. The methyl group at C-3 (secondary methyl group) is assigned a β-orientation on the basis of the following evidence.

A ^1H NMR solvent induced shift study of anhydroarthrosporone (21) was carried out. So-called aromatic solvent induced shifts (ASIS) are observed when the NMR spectrum of a compound is recorded in different solvents, one of which is inert (CCl_4) or pseudo-inert ($CHCl_3$), while the other is aromatic (C_6D_6 or C_5D_5N). The ASIS provides useful information about the stereochemistry of the chiral centres or groups of atoms in a molecule which contains site(s) capable of associating with the aromatic solvent. A π-π interaction is observed between a C=O group and π electron cloud of pyridine, while a hydrogen bonding interaction is observed between a hydroxyl group and the lone pair electrons on the nitrogen of pyridine. Such interactions induce anisotropy on the atoms near the site of aromatic solvent co-ordination. Assuming a 1:1 solute-pyridine complex is formed, the pyridine induced shift (PIS) for a given proton is expressed in terms of a difference between the shifts of the proton in $CDCl_3$ and C_5D_5N (equation below).

$$\Delta = \delta (CDCl_3) - \delta (C_5D_5N)$$

Δ in ppm is negative when the proton is deshielded by the anisotropic effect. The ASIS's have found application mostly in 6-membered ring compounds, especially steroids.[44] 1,3-Diaxial protons show large PIS (Δ is between −0.20 and −0.40 ppm) while the Δ value observed for *vicinal* protons is a function of the dihedral angle between the protons and the carbon oxygen bond.

Anhydroarthrosporone (21) contains several sites capable of bonding with pyridine. The most stable solute-pyridine complex will be formed at C-8 *via* hydrogen bonding between the OH and the lone pair electrons on the nitrogen atom (Structure A). The PIS will be extrapolated to anhydroarthrosporone. Table 3 shows that the C-1 proton *cis* to the OH is appreciably deshielded (Δ = -0.25). The methine proton at C-3 experiences a small pyridine induced shift (Δ = −0.09). This value is not large enough to draw conclusions concerning the stereochemistry of H-3 with respect to the hydroxyl group at C-8. Nevertheless the results of the solvent shift study have allowed the assignment of the chemical shifts of the methyl protons. The α methyl protons (H-12, pseudo 1,3-diaxial to the OH) experience a downfield shift. The ASIS of the ^1H NMR spectrum of anhydroarthrosporone (21) in C_5D_5N verifies the conclusions drawn from the acetate shifts



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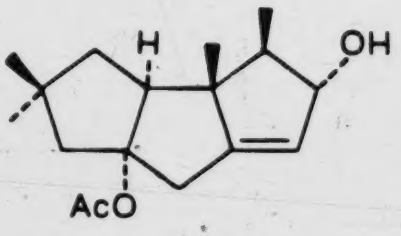


46

observed arising from the anisotropic effect of the acetoxyl group on the neighboring *cis* hydrogen in the ^{1}H NMR of anhydroarthrosporone (**21**) and its acetate **34**. The results of the solvent shift studies do not allow conclusions to be drawn with regard to the stereochemistry at C-3.

Reduction of the C-4 ketone to hydroxyl will provide a derivative in which the pyridine complexation should provide evidence regarding the stereochemistry at C-3. It was decided to protect the C-8 hydroxyl group to insure that the hydrogen bonding interaction only takes place at C-4. Acetate **34** was therefore prepared and subjected to a selective reduction using $NaBH_4$-$CeCl_3$ in methanol at 0°C.[47] A complex mixture of alcohols was obtained. The composition of the mixture was not affected by the reaction temperature or the order of addition of the reagents. Approximately the same mixture of components was obtained in the absence of cerium chloride. Three alcohols were isolated. The most polar alcohol was also the major product (*ca.* 70%), the least polar alcohol was produced in 15% yield while the alcohol of intermediate polarity was obtained in about 5% yield. The major and the minor alcohols produced identical colour reactions when subjected to the reagent A charring technique. They instantaneously give a red spot

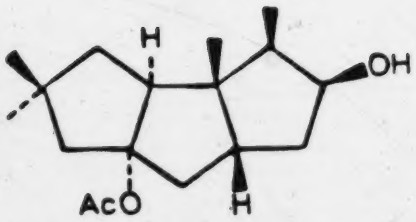
Table 3. ^1H NMR Spectral Data for Anhydroarthrosporone (400 MHz)

	δ, multiplicity (J values in Hz)		
H No.	**$CDCl_3$**	**C_5D_5N**	**Δ**
H-1	2.38, brt (10)	2.63, brt (10)	-0.25
H-3	2.32, q (7)	2.41 (q (7.5)	-0.09
H-5	5.85, brd (1.3)	5.92, d (2)	-0.07
H-12	1.22, s	1.40, s	-0.18
H-13	1.13, s	1.10, s	0.03
H-14	0.94, s	0.86, s	0.08
H-15	1.11, d (7)	1.14, d (7.5)	-0.03

which turns blue overnight. The third alcohol shows a different visualization reaction (bluish-grey spot) with reagent A. This alcohol was assigned structure **46** on the basis of the following evidence. The IR spectrum of **46** displays a broad hydroxyl absorption at 3400 cm^{-1}, strong bands characteristic of an acetoxyl group at 1732 and 1242 cm^{-1}, and weak C=C absorption band at 1674 cm^{-1}.

The HRMS shows the molecular ion at m/z 278 (M^+) corresponding to a formula $_9C_{17}H_{26}O_3$. The base peak in the mass spectrum appears at m/z 218 (M^+ -60) arising from the loss of acetic acid.

The ^1H NMR spectrum shows a vinyl proton at δ 5.29 (broad singlet). A carbinyl proton appears as a multiplet at δ 4.56. An AB spin system appears at δ 3.02 and 2.33 (J_{AB} = 17 Hz) with the proton at δ 2.33 showing allylic long-range coupling (^4J = 2.2 Hz) to the vinylic proton. The methine proton at C-3 appears as a doublet of quartets with J = 6 and 7.4 Hz respectively. A pyridine shift study led to the assignment of an α-configuration for the hydroxyl group at C-4. When the ^1H NMR of **46** is determined in pyridine, the proton at C-3 displays a pronounced pyridine induced shift (Δ =



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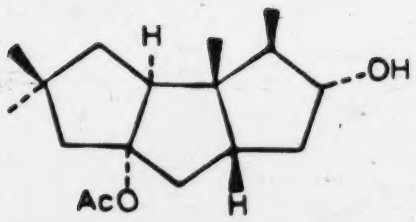


48

$\delta_{CDCl_3} - \delta_{C_5D_5N}$ = -0.46 ppm) while the β-oriented methyl group at C-2 shows no appreciable shift. Table 4 illustrates the deshielding effect of pyridine on the protons associated with ring C of the acetoxy alcohol **46**.

The results are in good agreement with the proposed structure and assigned stereochemistry. It is interesting that the methyl doublet (H-15) displays a significant downfield shift (Δ = -0.17 ppm) which may be explained on the basis of conformational considerations. Ring C of the triquinane system may adopt an envelope (C_s) conformation with C-3 being the flap of the envelope. The C-4 hydroxyl group lies between the methine and the methyl group at C-3, the dihedral angle θ (Figure 4) between the two groups then determining the extent of the deshielding. The smaller the dihedral angle θ, the larger is the deshielding effect.

The two alcohols which show identical colour reaction on TLC are diastereoisomers and are assigned structures **47** and **48** on the basis of the following evidence.

The IR spectrum of **47** displays a broad hydroxyl absorption at 3464 cm^{-1}, and a strong ester carbonyl absorption at 1732 cm^{-1} with a shoulder at 1716 cm^{-1}. The

Table 4. ^1H NMR Spectra Data for Compound 46 (400 MHz).

	δ, multiplicity (J values in Hz)		
	$CDCl_3$	C_5D_5N	Δ
H-3	1.80, dq (6, 7.4)	2.26, dq (6, 7.4)	-0.45
H-4	4.56, m	4.94, m	-0.38
H-5	5.29, brs	5.66, brd (2)	-0.37
H-14	0.80	.83	-0.03
H-15	1.09, d (7.5)	1.26	-0.17

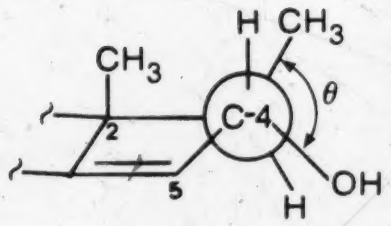


Figure 4: Newman Projection Down the C-4, C-3 Bond of Ring C.

molecular formula was established as $C_{17}H_{28}O_3$ (MW – 280) on the basis of a M+18 peak at m/z 298 in the CI of **47**. The ^1H NMR of the alcohol **47** shows a carbinyl proton at δ 4.31 as an apparent triplet of doublets (J = 6 and 3 Hz). Decoupling experiments show that this proton is coupled to *vicinal* protons at δ 2.37 – 2.30 (multiplet), 1.96 and 1.88. The 2.37-2.30 signal integrates for two protons. However, irradiation of the doublet methyl at δ 1.05 leads to a simplification of the signal at δ 2.37-2.30, thus one of the protons in this region is the methine proton at C-3. Once again a pyridine induced shift study was useful in assigning the ^1H NMR spectrum of compound **47**. A partial assignment of the spectrum is shown in Table 5.

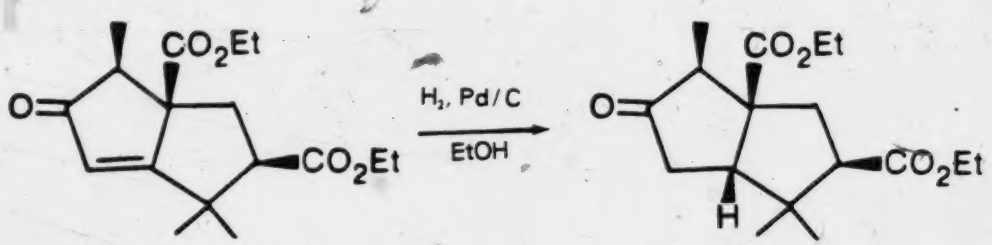
Table 5 shows that one of the methylene protons at C-5, the methyl singlet at C-2 and the methyl doublet (H-15) experience appreciable deshielding when the solvent is changed from $CDCl_3$ to C_5D_5N. The chemical shift of the C-3 methine (H-3) remains unchanged from one solvent to another. These observations agree well with the proposed relative stereochemistry at C-2, C-3 and C-4 in the acetoxy alcohol **47**. These data are in agreement with a *cis, anti, cis* configuration of the triquinane system. The reduction of the C-C double bond would be expected to take place from the top side (less sterically hindered face) of the molecule. For example, compound **49** upon catalytic hydrogenation gives the *cis* bicyclic ketone **50**. Compound **50** was also obtained by lithium ammonia reduction of **49**.[41]

The IR spectrum of the major alcohol (**48**) displays the same features as does the minor isomer **47**. The spectrum shows a hydroxyl absorption band at 3464 cm^{-1} and acetoxyl absorption bands at 1732 and 1243 cm^{-1}. The carbonyl absorption at 1732 cm^{-1} again has a shoulder at 1713 cm^{-1}.

The molecular formula $C_{17}H_{28}O_3$ (Mol. Wt. 280) was deduced on the basis of high resolution mass spectra (HRMS). Fragment ions at m/z 238 ($C_{15}H_{24}O_2$, $M^+ - CH_2CO$), 220 ($C_{15}H_{22}O$, $M^+ - HOAc$), and 202 ($C_{15}H_{26}$, $M^+ - (HOAc + H_2O)$ are observed. The ^1H NMR spectrum ($CDCl_3$) of major alcohol **48** shows a low-field proton at δ 3.89 as an apparent triplet of doublets with coupling constants J = 9 Hz and 6 Hz, respectively. The spectrum exhibits a methyl doublet at δ 1.03 and methyl singlets at δ 2.02 (CH_3CO), 1.05 (6 H) and

Table 5: ^1H NMR Spectral Data for Alcohol 47 (400 MHz)

	δ, multiplicity (J in Hz)		
	$CDCl_3$	C_5D_5N	Δ
H-3	2.34, m	2.34, qd (8, 4)	0.00
H-4	4.31, td (6,3)	4.52, m	-0.21
H-5a	1.81, m	2.13, ddd (13.5, 8, 3)	-0.32
H-5b	1.96, ddd (14, 6, 3.6)	2.01, ddd (14.2, 6, 4)	-0.04
H-14	0.95, s	1.21	-0.26
H-15	1.00, d (7.2)	1.25, d (8)	-0.25



49 **50**

0.80. The methyl doublet (J = 7.2 Hz) is coupled to a one proton doublet of quartets (J = 9 and 7.3 Hz) at δ 1.97.

The spectrum in pyridine was readily interpreted and allowed the assignment of most of the protons. Comparison of the spectra of **48** in $CDCl_3$ and C_5D_5N allowed the assignment of the relative stereochemistry at C-3 and C-4 (Table 6).

The H-3 methine shows a downfield shift (Δ = -0.41 ppm), while the chemical shift of the C-2 angular methyl is not affected by the solvent change. Therefore the C-4 hydroxyl group is α oriented. These results agree well with the proposed structure and stereochemistry at C-3 and C-4. The pyridine shift studies show that the methyl doublet at C-3 undergoes a substantial downfield shift (Δ = -0.19 ppm). Since the C-3 methyl has a β orientation, ring C of the triquinane system must again adopt an envelope conformation. Examination of molecular models reveals that the C-4 hydroxyl group is between H-4 and the C-3 methyl when C-3 assumes the flap position of the envelope. A Newman projection of the conformation viewed down the C-4, C-3 single bond is shown in Figure 5 below.

The negligible PIS of the angular C-2 methyl (H-14) supports an α orientation of the C-4 hydroxyl group. Further experiments were undertaken to confirm structure **48**. Since the major alcohol resulted from 1,4-hydride addition to unsaturated acetate **34** followed by the reduction of the resulting ketone **54**, preparation of the diol **52** *via* two successive reductive reactions was undertaken. Ketone **51** was obtained *via* catalytic hydrogenation of anhydroarthrosporone (**21**). Ketone **51** was then subjected to hydride reduction to give diol **52** (Scheme IX). The major alcohol thus obtained was compared with compound **53**, the deacetylation product of acetoxyalcohol **48** (Scheme X). Hydrogenation[41] of anhydroarthrosporone (**21**) over 10% palladium on charcoal in acidified methanol for 30 min at room temperature, gave a UV inactive ketoalcohol. The ketoalcohol was recrystallized from a Skellysolve B / diethyl ether mixture to give analytically pure needles m.p. 115-116°C. The spectroscopic data is in agreement with the proposed structure **51** for the reduction product. The IR spectrum of **51** shows strong bands at 3502 (OH) and 1727 (C=O) cm^{-1}.

The molecular ion at m/z 236 in the HRMS of **51** agrees with the molecular formula $C_{15}H_{24}O_2$. The ^1H NMR spectrum of ketoalcohol **51** shows singlet methyl signals at δ 1.14, 1.07 and 0.77. A methyl doublet (J = 7 Hz) at δ 0.99 is coupled to a broad quartet

Table 6: ^1H NMR Spectra Data For Alcohol 48 (400 MHz)

	δ, multiplicity (J in Hz)		
	$CDCl_3$	C_5D_5N	Δ
H-3	1.97, dq (9, 7.3)	2.38, dq (8, 7)	-0.41
H-4	3.89, td (9, 6)	4.18, m	-0.29
H-5a	1.38, ddd (13.2, 6, 3.5)	1.75, ddd (14.5, 6, 4)	-0.37
H-5b	2.29, dt (13.8)	2.04, ddd (15, 8, 4)	0.25
H-14	0.80, s	0.83, s	-0.03
H-15	1.03, d (7.2)	1.22, d (7)	-0.19

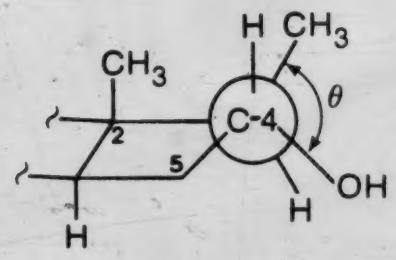
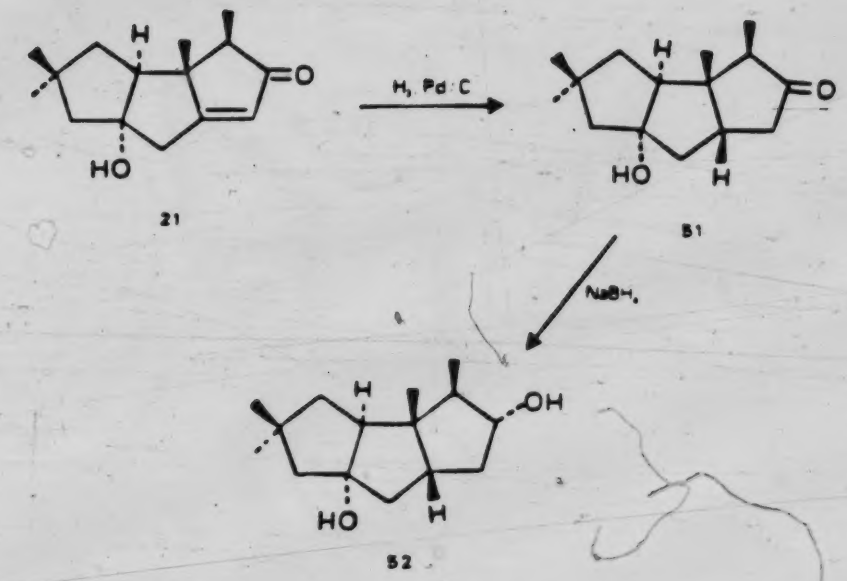


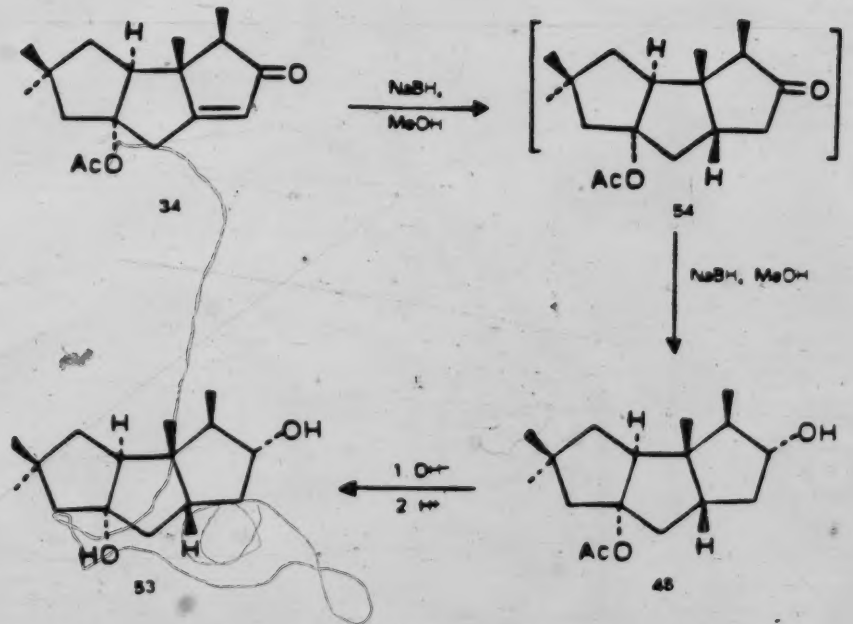
Figure 5

Scheme IX



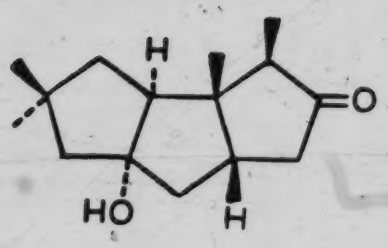
21 51 52

Scheme X



34 54 48 53

at δ 2.54 assigned to the H-3 methine proton. The δ 2.54 region integrates for two protons. The second proton appears as a broad doublet of doublets (J = 12.5 and 7 Hz) and is assigned to the H-1 methine. The C-5 methylene protons α to the carbonyl appear at δ 2.39 and 2.19. Each signal shows *geminal* coupling (J = 18 Hz) and the proton at δ 2.39 shows a *vicinal* coupling (J = 8 Hz) to the C-6 methine (multiplet at δ 2.31).



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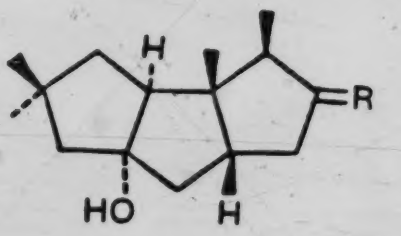


52 R =

55 R =

When ketoalcohol **51** was allowed to stir at room temperature in methanol containing sodium borohydride, two diastereoisomeric diols were produced. Both diols, when subjected to the reagent A charring technique on TLC, display the same color reaction as observed for acetoxyalcohols **47** and **48**.

The most polar diol was formed as the major product (yield > 85%). The major product was assigned structure **52** on the basis of its physical characteristics.

Diol **52** is a cotton-like solid (m.p. 139-140°C). Its IR spectrum exhibits strong and broad OH absorptions at 3345 cm^{-1} (broad, OH) and 1040 cm^{-1} (C-O). The molecular weight as determined by CI is 238, consistent with the formula $C_{15}H_{26}O_2$. Peaks in the mass spectrum at m/z 220 ($C_{15}H_{24}O$, $M^+ - H_2O$) and 202 ($C_{15}H_{22}$, $M^+ - 2H_2O$) are indicative of the presence of two hydroxyl groups. The ^1H NMR spectrum of diol **52** displays only one

downfield proton (H-4) at δ 3.84. This proton appears as an apparent triplet of doublets with J = 9 and 6.5 Hz. The spin pattern which has previously been observed in the ^{1}H NMR spectrum of **52** consists of fairly well resolved spin systems. Extensive decoupling experiments allowed the assignment of all the protons in the molecule (see Experimental).

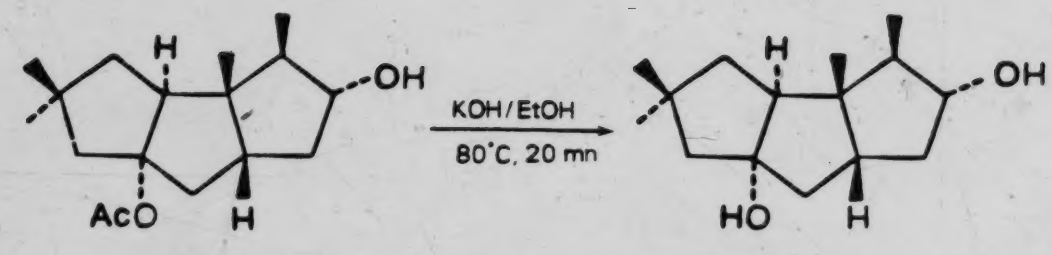
The minor diol was assigned structure **55** on the basis of its ^{1}H NMR. Its only downfield proton (H-4) appears at δ 4.29 as a triplet of doublets (J = 6 and 2.5 Hz). This chemical shift and the decoupling constants are very similar to the values observed in the ^{1}H NMR spectrum of acetoxyalcohol **47** (δ 4.31, td, J = 6 and 3 Hz). The C-2 methyl group in the diols resonate at δ 0.93 in the case of **55** and at δ 0.78 in the case of **52**, suggesting that the C-4 β hydroxyl group deshields the β angular methyl group (H-14) more than the δ hydroxyl group, as would be expected.[50]

Final correlation of compounds **52** and **48** was provided by conversion of **48** into **52**. When acetoxyalcohol **48** was allowed to stir at 80°C in 10% ethanolic potassium hydroxide,[51] diol **53** was obtained in good yield as the sole product. Compound **53** was identical in all aspects (TLC, IR, MS and ^{1}H NMR) with compound **52**.

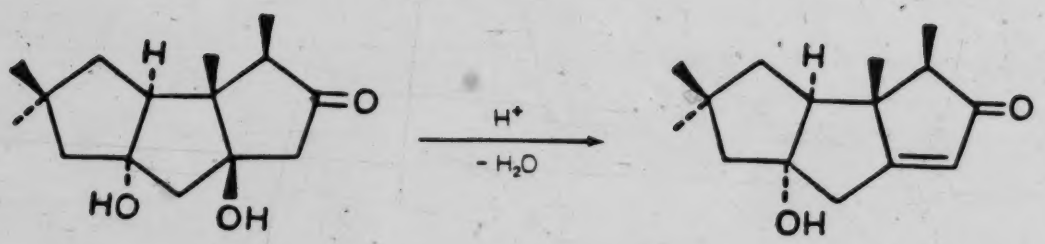
Structure and stereochemistry of arthrosporone (20)

Since arthrosporone (**20**) readily dehydrates under acid catalysis[52] to anhydroarthrosporone (**21**) (see Experimental) structure **28** was unequivocally assigned to be that of arthrosporone (**20**). The *cis* AB ring junction in **21** as well as the configuration at C-2 has been established. The stereochemistry at C-3 and C-6 in **20** were assigned on the basis of the following evidence.

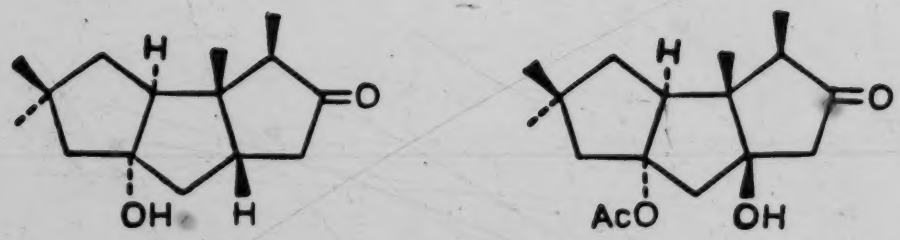
The stereochemistry of the C-3 methyl of arthrosporone should be the same as that in anhydroarthrosporone provided that no isomerization took place during the dehydration reaction. Evidence that the stereochemical integrity at the center is retained is provided by ^{1}H NMR data: the H-3 methine resonates at δ 2.57 in arthrosporone (**20**) and at δ 2.54 in the reduction product **51** obtained by catalytic hydrogenation of anhydroarthrosporone (**21**). This indicates that the methine proton at C-3 in both **20** and **51** is in a similar environment.



50 53

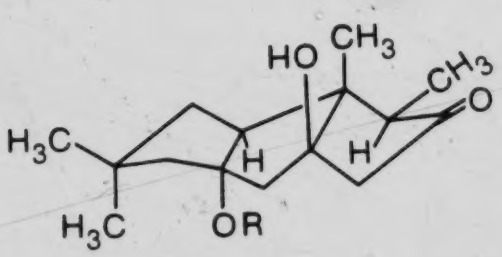


28 ≡ 20 21



51 27

Examination of the ^1H NMR spectra of arthrosporone (**20**) and O-acetylarthrosporone (**27**) reveals that the H-3 proton moves upfield when the C-8 hydroxyl group is acetylated. This unusual shielding ($\Delta\delta$ = 0.22 ppm) in monoacetate **27** is explained if the H-3 methine and C-8 functional group are proximate to one another. Examination of molecular models reveals that they are close to one another in space (a conformational drawing of **27** is shown below).



27 R = Ac

Results of the ^1H NMR solvent shift studies with arthrosporone and 8-O-acetyl-arthrosporone are presented in Tables 7 and 8 and lend support to the stereochemical assignment. Table 7 shows that in **20** H-3 is deshielded (Δ = -0.35 ppm) when the ^1H NMR spectrum recorded in pyridine is compared with that recorded in $CDCl_3$. The C-2 methyl group (H-14) shows moderate deshielding (Δ = -0.10 ppm) while the C-3 methyl group (H-15) is not shifted. Similar solvent shifts are observed for 8-acetoxyarthrosporone (Table 8).

The negligible anisotropy experienced by the C-3 β methyl (Tables 7 and 8) may be explained by the fact that ring C assumes an envelope conformation with C-3 at the flap (Figure 6).

Table 7. ^1H NMR Spectral Data for Arthrosporone (400 MHz)

	δ, multiplicity (J values in Hz)		
	$CDCl_3$	C_5D_5N	Δ
H-1	2.56, dd (12, 8)	2.77, dd (12, 9.5)	-0.21
H-3	2.57, brq (7)	2.92, qd (7, 1)	-0.35
H-12	1.16, s	1.24, s	-0.08
H-13	1.08, s	0.96, s	0.12
H-14	0.84	0.94, s	-0.10
H-15	1.02, d (7)	1.00 (d (7)	0.02

Table 8. ^1H NMR Spectral Data of O-Acetylarthrosporone 27

	δ, multiplicity (J value in Hz)		
	$CDCl_3$	C_5D_5N	Δ
H-5a	2.64, dd (19, 1)	2.69, brd (19)	-0.05
H-5b	2.22, bd (19)	2.44, brd (19)	-0.22
H-7a	2.81, d (16)	3.10, brd (16)	-0.29
H-7b	2.24, d (16)	2.45, brd (16)	-0.21
H-12	1.04, s	1.01, s	0.03
H-14	0.88, s	1.09, s	-0.21
H-15	1.06, d (7)	1.11, d (7)	-0.05

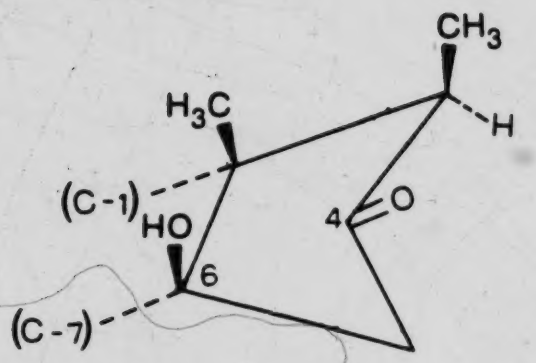
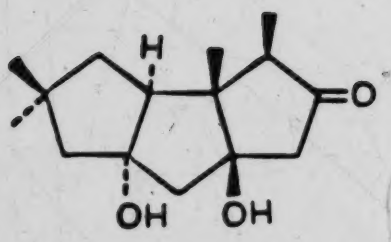


Figure 6: Ring C viewed from the top.

Attempted base-catalyzed benzoylation[53] of arthrosporone led to recovery of arthrosporone (**20**) along with a small amount of a slightly more polar compound **57** (TLC, acetone / chloroform 3:7). Compound **57** was produced in larger quantity by treatment of arthrosporone with a catalytic amount of DMAP and a few drops of Et_3N in refluxing dichloromethane. An apparent equilibrium mixture of **20** and **57** in the ratio of 10:1 was obtained after 24 h. The mixture was separated and the starting material resubjected to the reaction conditions. This procedure was repeated several times. In this way about 2 mg of **57** was obtained from **20** (6 mg).

Compound **57** was found to be an isomer of arthrosporone (epimeric at C-3) and thus is named *iso*arthrosporone. Its spectroscopic data are consistent with structure **57** in

which the C-3 methyl is in the α configuration.



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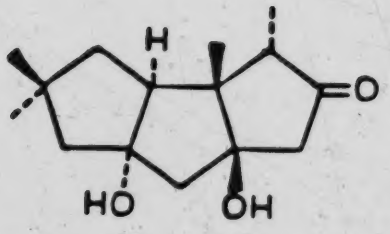
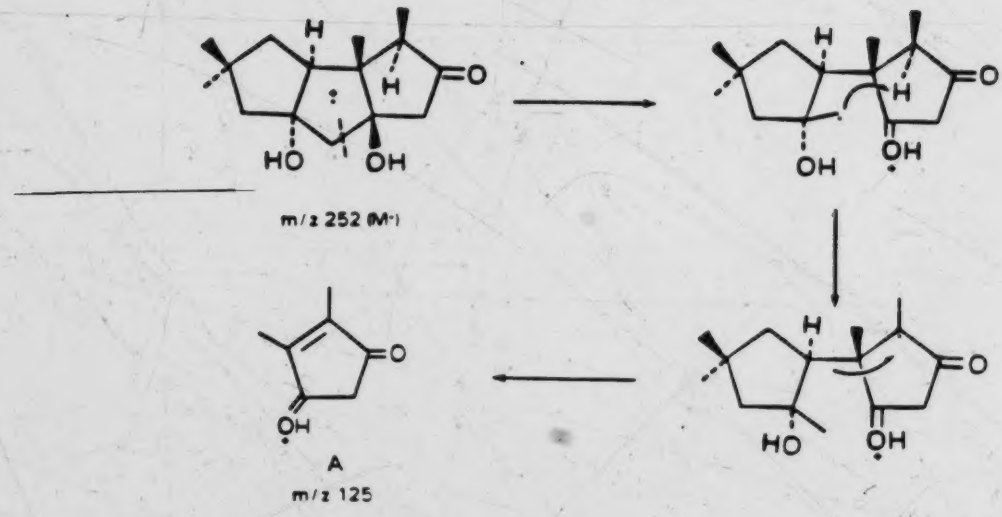


57

Compound **57** is a waxy solid. It possesses a molecular weight of 252 ($C_{15}H_{24}O_3$) as evidenced by HRMS. The fragmentation pattern in its MS is identical with that of arthrosporone (**20**) except for the fragment representing the base peak. The base peak in **20** appears at m/z 125 ($C_7H_9O_2$) while in **57** the base peak appears at m/z 83 (C_5H_7O). The two fragments are postulated to have structures A and B, respectively. A may arise as shown in Scheme XI. The fact that this may not be facile in the *iso* series may lead to the fragmentation shown in Scheme XII below. Both isomers **20** and **57** are optically active. Arthrosporone (**20**) has a specific rotation of -140° while isoarthrosporone (**57**) has a rotation of +38°. The IR spectrum of **57** is similar to that of **20** (3435 cm^{-1} (OH), 1728 cm^{-1} (C=O)). One distinguishing difference between arthrosporone (**20**) and *iso*arthrosporone (**57**) is shown in their ^1H NMR spectra. Table 9 summarizes the main differences in chemical shifts of compounds **57** and **20**. The C-1 and C-3 methines, the C-5 methylene protons and the C-2 angular methyl (H-14) show the greatest differences in the ^1H NMR spectra of compounds **20** and **57**.

Scheme XI



Scheme XII

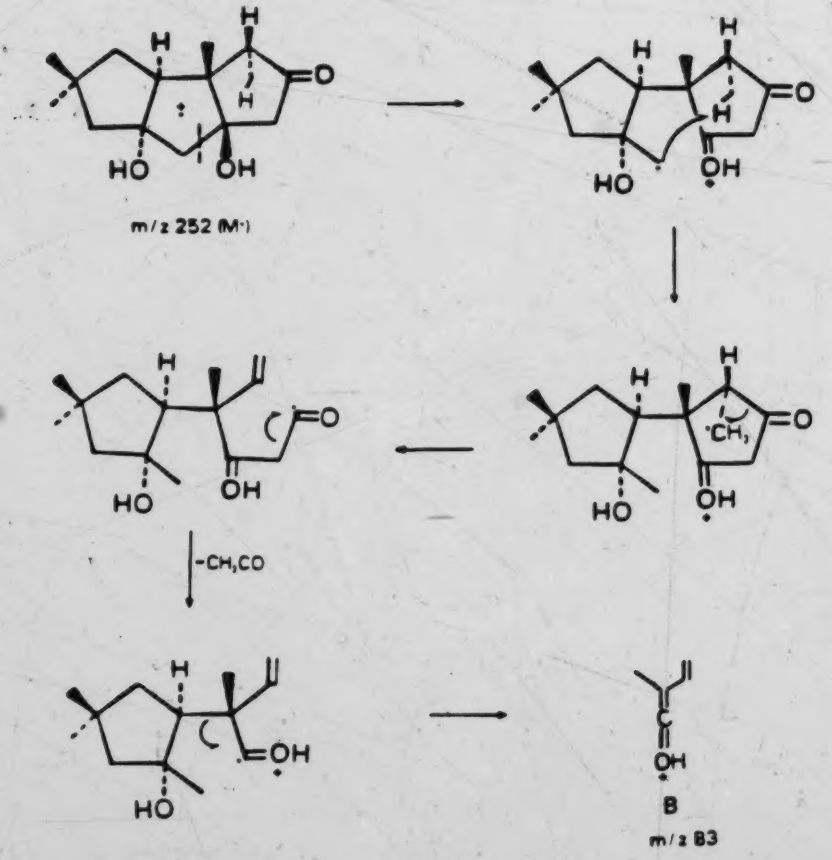


Table 9: ^1H NMR Spectral Data for Arthrosporone and *Iso*arthrosporone ($CDCl_3$, 400 MHz)

	δ (multiplicity, J values in Hz)	
	Arthrosporone	*Iso*arthrosporone
H-1	2.56 (dd, 12, 8)	2.10 (brt, 9.5)
H-3	2.57 (brq, 7)	2.29 (qd, 7, 1.5)
H-5a	2.69 (dd, 19.8, 1)	3.17 (d, 20)
H-5b	2.20 (d, 19.8)	2.46 (dd, 20, 1.5)
H-12	1.16, s	1.18, s
H-13	1.08, s	1.09, s
H-14	0.84, s	1.04, s
H-15	1.02 (d, 7)	1.00 (d, 7)

Table 10. ^1H NMR Spectral Data for Isoarthrosporone (400 MHz)

	δ, multiplicity (J values in Hz)		
	$CDCl_3$	C_5D_5N	Δ
H-1	2.10, brt (9.5)	2.44, brt (9)	-0.34
H-3	2.29, qd (7, 1.5)	2.56, brq (7.2)	-0.27
H-12	1.18, s	1.35, s	-0.17
H-13	1.09, s	1.08, s	0.01
H-14	1.04	1.25, s	-0.21
H-15	1.00	1.12, d, (7.2)	-0.12

The α-orientation of the C-3 methyl (see Table 9) in **57** accounts for the shielding of the C-1 proton and the deshielding of one of the C-5 protons and the C-14 methyl* group observed in the ^{1}H NMR of **57** with respect to the ^{1}H NMR of **20**. The β-oriented H-3 methine is shifted upfield due to its proximity to the C-6 hydroxyl group. Results of ^{1}H NMR solvent shift studies with *iso*arthrosporone are given in Table 10. The observed pyridine induced shifts (PIS) are consistent with the assigned stereochemistry at C-3 in *iso*arthrosporone (**57**). These studies show the deshielding of H-3 (Δ = -0.27 ppm), the C-2 methyl (Δ = -0.21 ppm), and the C-3 methyl (Δ = -0.12). The deshielding of the C-3 methyl is due to its spatial proximity to the C-8 hydroxyl group.

We hoped that the ^{1}H NMR spectrum of arthrosporone diacetate **58** would provide confirmation of the stereochemistry of the C-6 and C-8 hydroxyl groups in arthrosporone. *Trans* 1,3-hydroxyl groups would be expected to induce a simultaneous acetoxyl anisotropic shift on both C-7 methylene protons. The ^{1}H NMR spectrum of **58** is shown in Table 11. One of the C-7 protons is strongly deshielded relative to arthrosporone monoacetate (**27**), the other is slightly shielded. The origin of the shielding effect on the C-7 protons is not clear and the measurements were not helpful in the confirmation of the stereochemistry.

It has been shown that arthrosporone (**20**) can be readily dehydrated to anhydroarthrosporone (**21**) under acid catalysis. Several attempts have been made to hydrate anhydroarthrosporone to produce compound **20** and the results are reported below.

We felt that epoxidation of **21** to give **59** followed by reductive opening of the epoxide (Scheme XIII) was one possible approach to arthrosporone (**20**). It was expected that epoxidation would provide the thermodynamically more stable *cis,anti,cis* triquinane. Dissolving metal reduction should then give arthrosporone (**20**).

Attempted epoxidation of compound **21** using known procedures for the epoxidation of other hirsutane sesquiterpenes[53] was unsuccessful. However, in one case

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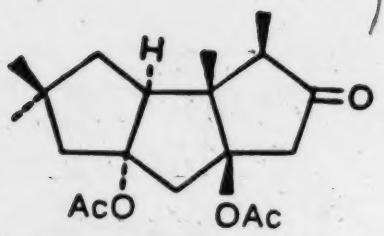
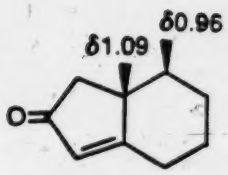
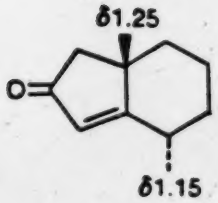
*The effect of a *cis* methyl group on the chemical shift of an angular methyl is shown in compounds I and II.[54]



58



i

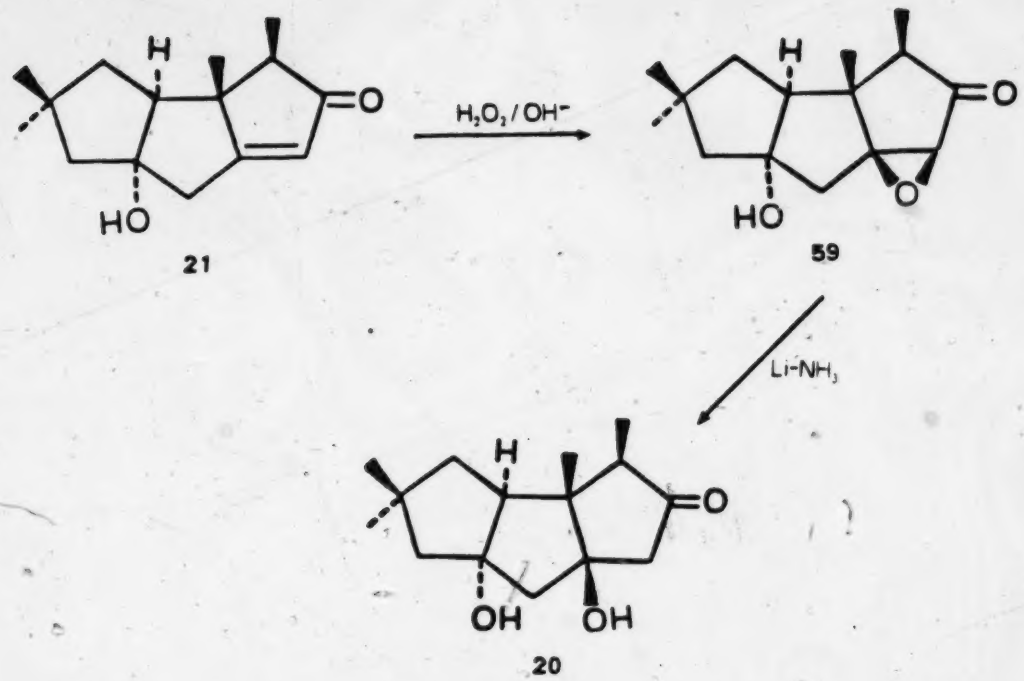


ii

Table 11. ^{1}H NMR Spectral Data for Monoacetoxyarthrosporone (27) and Diacetoxyarthrosporone (58)

	δ ($CDCl_3$), multiplicity (J in Hz)	
	Compounds	
	27	**58**
H-1	2.83, dd (8.5, 12)	2.86, dd (8, 12)
H-5a	2.64, dd (19, 1)	3.21, dd (19, 1)
H-5b	2.20, d (19)	2.29, d (19)
H-7a	2.8a, d (16)	3.30, d (17)
H-7b	2.24, d (16)	2.18, d (17)
H-14	0.88, s	0.88, s
H-15	1.06, d (7)	1.05, d (7)

Scheme XIII



21 59 20

in which the reaction mixture was stirred at room temperature for one day, a mixture of compounds was obtained. TLC showed the presence of starting material **21** along with several other compounds. Column chromatography of the reaction mixture led to the isolation of an unidentified compound $C_{15}H_{22}O_4$ (for spectral details see Experimental) and a relatively non-polar crystalline material **60** (m.p. 115-117°C).

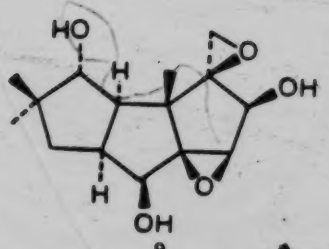
The molecular weight of compound **60** was shown to be 250 ($C_{13}H_{22}O_3$) as evidenced by CI (268, 100%, M+18). The HRMS of **60** shows fragment ions at m/z 207 ($M^+ - CH_2CO - H$), m/z 204 ($M^+ - CO - H_2O$), and the base peak at m/z 109 (C_7H_9O).

The IR spectrum displays a hydroxyl absorption (3464 cm^{-1}), a carbonyl absorption (1749 cm^{-1}, strong) and CO absorption bands (1180, 1142 and 1036 cm^{-1}). Compound **60** possesses five sites of unsaturation, probably a five-membered ring ketone, and at least one hydroxyl group.

The ^1H NMR spectrum of **60** shows a series of one-proton signals. There is no downfield signal indicative of vinylic or carbinylic protons.[54] An AB spin system (δ 2.78, 2.40, J = 18 Hz) is indicative of geminal methylene protons on a carbon α to a C=O group. The chemical shifts of the system are close to that observed in the ^1H NMR of arthrosporone. The proton at δ 2.40 was shown to have a long range coupling (^4J = 2 Hz) to one proton of another AB spin system (J = 14 Hz) at δ 2.31 (apparent doublet of doublets) and δ 2.13 (broad doublet). The remainder of the ^1H NMR spectrum displays a coupling pattern similar to that observed in arthrosporone (**20**) and anhydroarthrosporone (**21**). The distinctive feature in the ^1H NMR spectrum of **60** is the absence of the methyl doublet and methine quartet (observed at C-3 of anhydroarthrosporone (**21**)). There are four methyl groups (all singlets of δ 1.18, 1.15, 1.14 and 1.06). Structure **60** is tentatively proposed for this compound. Molecular models show that the β-proton at C-5 and the β-proton at C-7 have a W arrangement, accounting for the long range coupling observed. Scheme XIV suggests one possible mechanism for its formation. It is interesting that the ^1H NMR spectrum of **60** does not show the long range coupling (J = 2 to 3 Hz) usually observed between the C-9 and C-11 protons in the ^1H NMR spectrum of anhydroarthrosporone, arthrosporone and their derivatives.

Although anhydroarthrosporone failed to undergo epoxidation, two naturally occurring hirsutanes, coriolin (**9**), and hirsutic acid (**12**), which possess the 5β,6β epoxide are known. In addition it has been reported that ketodiene **61** is transformed into mono epoxide **62** in high yield.[56]

Both hirsutic acid (**12**) and compound **61** possess an exocyclic methylene group at C-3, while in coriolin (**9**) the C-4, C-15 bond is α-oriented such that any steric congestion between the C-2 methyl and the C-3 substituent is kept to a minimum. Anhydroarthrosporone possesses a hydroxyl group at C-8. Examination of molecular models of the arthrosporone series shows that the C-8 OH is in relatively close proximity to any large group (i.e., methyl) which has an α-orientation at C-3. In anhydroarthrosporone the C-3 methyl is β. The β epoxide derivative, if formed, will be destabilized by the two β-methyl groups at C-2 and C-3. On the other hand the α-epoxide cannot be formed since



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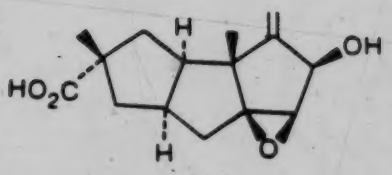

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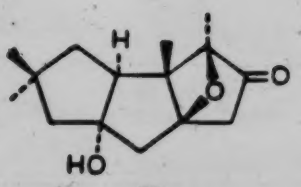
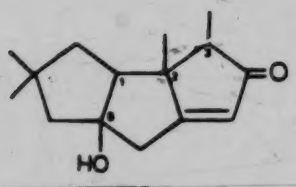


60

Scheme XIV



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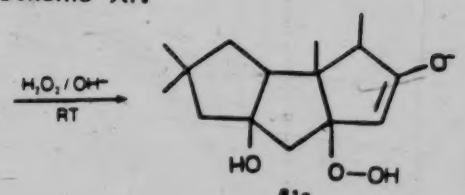
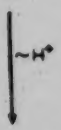
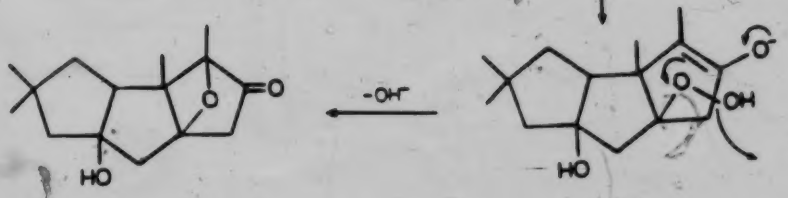
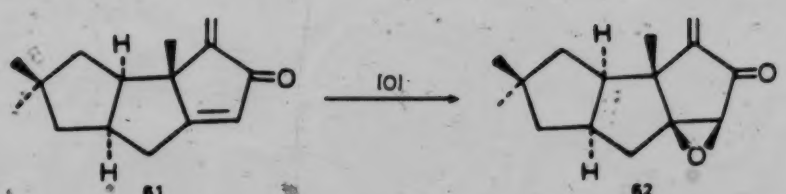


61a





60 61b



61 62

a thermodynamically less favorable *cis,anti,trans* triquinane will be formed. Thus anhydroarthrosporone did not undergo a facile epoxidation. Under forcing conditions, a

more complex reaction occurred. Inversion of configuration at C-3 and formation of the C-3, C-6 four-membered ring ether took place.

Arthrosporol (22)

Arthrosporol is the third most abundant metabolite isolated by chromatography of the Arthrosporae broth extract. It is a very polar compound (R_f* 0.39 (acetone / benzene 3:2), 0.32 (acetone / benzene 2:3, 2xdevelopment) which chars red after visualization with reagent A.

The IR spectrum of **22** displays a strong and broad hydroxyl absorption (3376 cm^{-1}), sharp and strong C-H stretching bands (2951 and 2933 cm^{-1}), and bands characteristic of *gem*-dimethyl C-H bending vibrations (as a doublet, 1380 and 1372 cm^{-1}).

The compound has a molecular weight of 254 (CI m / z 272, 100%, $M+NH_4^+$, 254, 57%, M^+). The HRMS of arthrosporol shows a peak at m / z 236 ($C_{15}H_{24}O_2$, $M^+ - H_2O$) and a base peak at m / z 218 ($C_{15}H_{22}O$, $M^+ - 2H_2O$). The mass spectral results indicate that arthrosporol has the molecular formula $C_{15}H_{24}O_3$.

The ^1H NMR spectrum (Figure 7) of arthrosporol shows a downfield proton (apparent doublet of triplets, J = 9 and 5 Hz) at δ 3.94 coupled to protons in the δ 2.06-1.96 region (multiplet, 3 protons). Decoupling experiments reveal several other one-proton spin systems represented by the proton coupling pattern shown in Scheme XV. Similar spin systems have been described for the previously discussed metabolites of Arthrosporae. The spectrum also exhibits two D_2O exchangeable protons (δ 1.77 and 1.64, both as broad singlets), three methyl singlets (δ 1.11, 1.04 and 0.76) and a methyl doublet (δ 1.02, J = 7 Hz) coupled to a proton in the δ 2.06-1.96 region.

The ^{13}C NMR of arthrosporol in CD_3OD shows two

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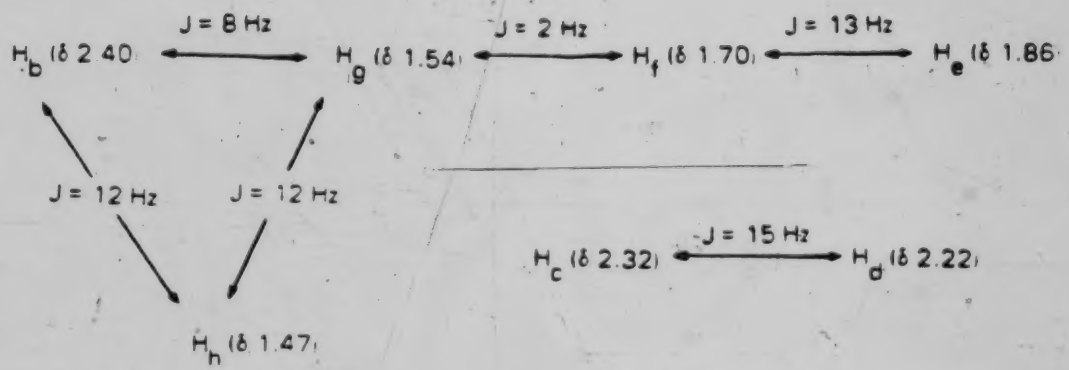
* R_f is relative R_f calculated using metabolite **21** as a reference.

fully substituted carbons bearing oxygen atoms at δ 91.0 and δ 90.0.[41] The peak at δ 76.8 (d) indicates the presence of a methine carbon bearing an oxygen atom.[37] The remainder of the spectrum consists of 2 singlets, 2 doublets, 4 triplets and 4 quartets. The ^{13}C NMR confirms the proposed formula $C_{15}H_{24}O_3$ (3 unsaturations) for arthrosporol. The absence of a sp^2 hybridized carbon[37] coupled with the presence of four methyl groups (4 quartets) and three carbons bearing oxygen atoms led to the conclusion that arthrosporol is a tricyclo-undecanoid triol. Thus structure **22** was tentatively proposed as that of arthrosporol.

Scheme XV. ^1H NMR Coupling Pattern of Arthrosporol (22)



The structural assignment for arthrosporol was confirmed by chemical transformations. When a solution of arthrosporol (**22**) in dichloromethane was allowed to stir overnight in the presence of pyridinium chlorochromate (PCC),[39] a single product **63** was formed m.p. 139-140°C, $[\alpha]_D$ -145° ($CHCl_3$), m/z calcd for $C_{15}H_{24}O_3$: 252.1719, found 252.1723 (M^+). Compound **63** was shown to be identical with arthrosporone (**20**) in all respects (TLC, IR, MS and ^1H NMR). Therefore, the structure of arthrosporol is that shown in **22**.

It was assumed that the stereochemistry of the C-3 methyl group was unchanged during the oxidation of arthrosporol (**22**) to arthrosporone (**20**). To verify this,

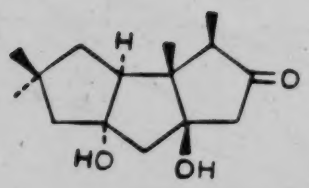
arthrosporone (**20**) was subjected to sodium borohydride reduction in methanol at room temperature for 1 h.[39] Two diastereomeric triols **64** and **65** were obtained. The major triol, **64**, which was the most polar triol, showed the same R_f and similar charring characteristics on TLC as arthrosporol. Separation of the triols and analysis of the spectroscopic properties of triol **64** led to the conclusion that this synthetic triol was identical in all aspects with naturally-occurring arthrosporol (**22**). Therefore the stereochemistry of the C-3 methyl of arthrosporol is the same as that of arthrosporone (**20**).

The stereochemistry of the hydroxyl group at C-4 was assigned as α on the basis of the evidence which follows. There exists a striking similarity in both the TLC behavior and the ^{1}H NMR spectra of arthrosporol (**22**), acetoxyalcohol **48** and diol **52**. All three alcohols display the same colour reaction on TLC when sprayed with 1% vanillin in sulfuric acid (reagent A) and heated. The chemical shifts and the spin systems of the C-4 carbinyl proton in the ^{1}H NMR of **22**, **48** and **52** are similar (Table 12).

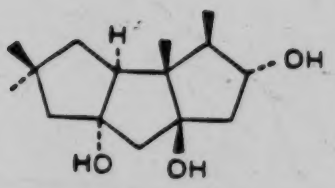
Since the stereochemistry in acetoxyalcohol **48** and diol **52** has already been established as α, the stereochemistry of the C-4 hydroxyl group in arthrosporol (**22**) can be deduced to be α.

To verify the stereochemistry assignment at C-4 in arthrosporol an analysis was made of the acetoxyl induced anisotropic shifts of protons *vicinal* to the hydroxyl groups in arthrosporol and its mono-, di- and tri-acetyl derivatives. The monoacetyl, diacetyl, and triacetyl derivatives of arthrosporol were prepared selectively as follows.

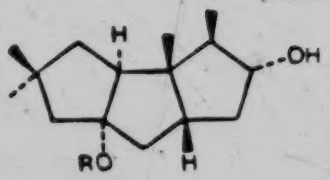
Arthrosporol monoacetate (**66**) was prepared by treatment of arthrosporol (**22**) with acetic anhydride in pyridine for 24 h or by treatment of **22** with acetic anhydride, a catalytic amount of DMAP, and triethylamine[40] for 3 hours. The latter reaction when allowed to proceed for 3 days gave a quantitative yield of arthrosporol diacetate (**67**). Arthrosporol triacetate (**68**) was obtained quantitatively when arthrosporol (**22**) was allowed to stir with a catalytic amount of *p*-toluenesulfonic acid in acetic anhydride[41] for 3 hours.



63 ≡ 20



22



48 R = Ac

52 R = H

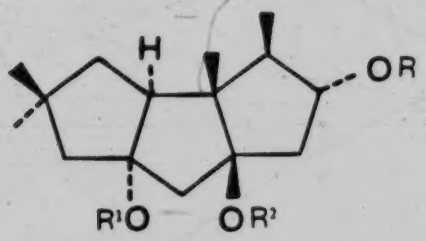
Table 12. ^{1}H NMR Spectral Data for Arthrosporol (22) Acetoxyalcohol 48 and Diol 52

	δ, multiplicity (J in Hz)		
	22	48	52
H-4	3.95, td (8, 6)	3.89, td (9, 6)	3.84, td (9, 6.5)
H-12*	1.11, s	1.05, s	1.12, s
H-13	1.03, s	1.05, s	1.04, s
H-14	0.77, s	0.80, s	0.78, s
H-15	1.02, d (7 Hz)	1.03, d (7 Hz)	1.00, d (7 Hz)

*H-12 to H-15 represent the methyl groups.

Each of acetates (66-68) is optically active. Monoacetate 66 is crystalline and decomposes at 150°C. The diacetate 67 is a waxy solid while the triacetate 67 is a viscous

oil. Monoacetate **66** is the most polar of the three derivatives of arthrosporol on TLC. Structures **66**, **67** and **68** were assigned to the arthrosporol mono-, di-, and triacetates, respectively, on the basis of spectroscopic data (see Experimental Section).



22 R = R¹ = R² = H (arthrosporol)

66 R = Ac, R¹ = R² = H

67 R = R¹ = Ac, R² = H

68 R = R¹ = R² = Ac

A characteristic feature appears in the IR ($CHCl_3$ cast) spectra (Table 13) of the three derivatives of arthrosporol (**22**). Table 13 shows that arthrosporol triacetate **68** displays an ester carbonyl stretching frequency at 1738 cm^{-1}, characteristic of acetates (1735 cm^{-1}).[62] The IR of arthrosporol diacetate (**67**) displays an ester carbonyl absorption at 1732 cm^{-1} with a shoulder at 1718 cm^{-1}.

Arthrosporol monoacetate (**66**) on the other hand, shows in its IR spectrum an ester carbonyl absorption at 1715 cm^{-1} with a shoulder at 1732 cm^{-1}. The carbonyl bands at 1715 and 1718 cm^{-1} are at unusually low frequency for an acetate carbonyl. Since compounds **66** and **67** possess two and one hydroxyl groups, respectively, intramolecular or intermolecular hydrogen bonding, between the carbonyl oxygen and the hydroxyl hydrogen may account for the low frequency of the carbonyl absorption.

If intramolecular H-bonding occurs, then the C-4 hydroxyl group in diacetate **67** would be expected to be β-oriented, whereas intermolecular hydrogen bonding favors the

Table 13. IR Spectral Data ($CHCl_3$ cast) for Arthrosporol Derivatives 66-68

	Frequency in cm^{-1}		
Function	Compounds		
	66	**67**	**68**
O-H	3456	3512	--
C=O	1732,* 1715	1732, 1718*	1738
C-O	1260, 1020	1249, 1020	1249, 1225, 1020

Table 14. IR Spectral Data for Arthrosporol Diacetate (67) in Carbon Tetrachloride Solution

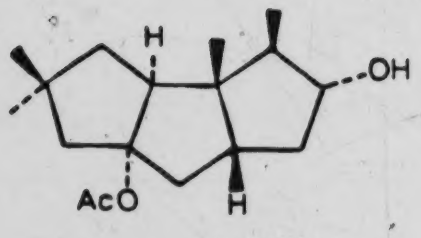
	Frequencies in cm^{-1}		
Function	Concentration		
	0.1 MM	0.05 MM	0.025 MM
O-H	3615	3615	3616
C=O	1733	1734	1734

*Appears as a shoulder.

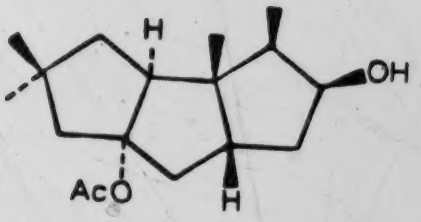
α-orientation of the C-4 hydroxyl group. An experiment which allows distinction between the two types of hydrogen bonding involves a study of the effects of dilution on the IR spectrum of **67**. The IR spectra of **67** were recorded in solution using carbon tetrachloride as solvent. The results, summarized in Table 14, show only one carbonyl absorption at 1734 cm^{-1}. The hydroxyl band sharpens and moves to a higher frequency. Therefore, the carbonyl absorption at 1718 cm^{-1} observed in the $CHCl_3$ cast IR spectrum of the diacetate **67** is due to an intermolecularly hydrogen bonded acetate.[63] This is in agreement with the *trans* relationship between the C-4 and C-6 hydroxyl groups in arthrosporol. The low frequency observed for the carbonyl of monoacetate **66**, much more pronounced than in the case of **67**, is probably due to an intramolecularly hydrogen-bonded acetate,[64] but dilution studies were not performed due to solubility problems. This is consistent with an α orientation of the C-4 hydroxyl group in arthrosporol (**22**). Inter- and intramolecular hydrogen bonding may also explain the presence of shoulders observed in the carbonyl absorption region in the film cast IR spectra of saturated acetoxyalcohols **47** and **48**. The ^1H NMR data (Table 15) of arthrosporol and its acetate derivatives provide additional evidence for the stereochemistry of the C-4 and C-6 hydroxyl groups. The acetoxyl induced shift is helpful in locating protons *cis* to the hydroxyl groups. The table shows that when there is an acetoxyl group at C-4 (e.g. **66, 67, 68**) a strong deshielding of the C-3 proton, as well as a deshielding of one of the C-5 protons, is observed. A large downfield shift of the C-4 proton is expected since the C-4 hydroxyl group is substituted with an acetoxyl group. The substitution of the C-8 hydroxyl group with an acetoxyl group (e.g. **66, 67**) results in a downfield shift of the C-1 (*vicinal* and *cis*) methine, one of the methylene protons at C-7, and one at C-9, as well as the C-10α methyl protons (H-12). The shielding of H-3 when an acetoxyl group is introduced at C-8 (**66→67**) is not surprising. The α-oriented H-3 is located close in space to the C-8 acetoxyl group and may be shielded by the latter. The shielding of the C-3 proton by the C-8 acetoxyl group has previously been observed in ^1H NMR of 8-acetoxyarthrosporone (**27**). The expected deshielding of both C-5 methylene protons is observed when all three hydroxylic protons are substituted with acetyl groups, consistent with the *trans* relationship between the C-4 and C-6 hydroxyls.

Table 15. ^1H NMR Spectral Data for Arthrosporol (22) and the O-Acetyl Derivatives (66-68), 400 MHz

	Chemical Shifts δ ppm ($CDCl_3$, 400 MHz)			
	22	**66**	**67**	**68**
H-1	2.40	2.42	2.70	2.75
H-3	2.01	2.22	2.03*	2.02
H-4	3.94	4.84	4.82	4.85
H-5	2.01	2.13	2.13	2.43
		1.95	1.88	2.30
H-7	2.32	2.27	2.64	3.19
	2.22	2.22	2.34	2.32
H-9	1.86	1.90	2.20	2.25
	1.70	1.71	1.82	1.53
H-12	1.11	1.12	1.03	1.01
H-14	0.78	0.79	0.81	0.84
H-15	1.02	0.99	1.00	0.99
CH_3CO	--	2.05	2.04	2.06, 2.03
			2.02	2.00

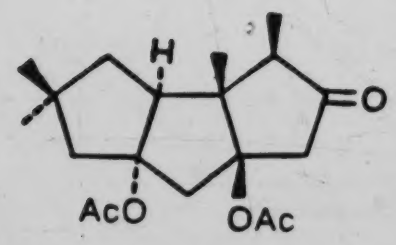


47



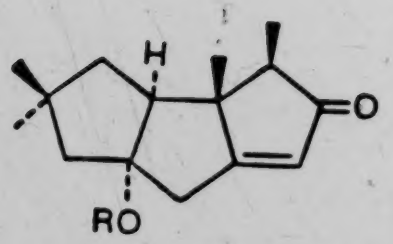
48

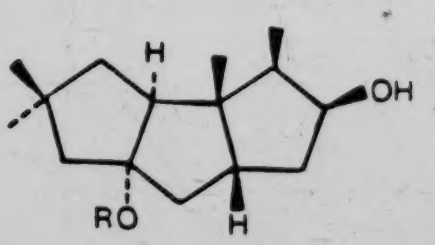
The ^{1}H NMR data for **68** shows a strong deshielding of only one of the C-7 protons. Since the C-6 and C-8 acetoxyl groups are *trans* it was expected that both C-7 protons would be deshielded. The cause of the large downfield shift of only one of the C-7 protons is not clear. This unusual deshielding has also been observed in ^{1}H NMR spectrum of arthrosporone diacetate (**58**).



58

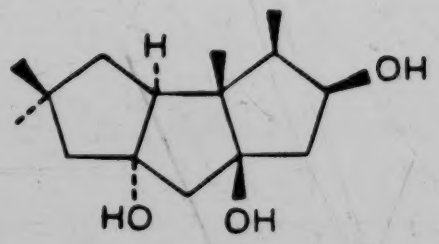
Reduction of arthrosporone also produced a minor triol **65** which shows the same TLC behavior as that of compounds **47** and **55** obtained by reduction of anhydroarthrosporone acetate (**34**) and anhydroarthrosporone (**21**), respectively. The minor triol, which we name *epi*arthrosporol, was assigned structure **65** on the basis of spectroscopic data.





R = H **21** **55**

R=Ac **34** **47**



65

Table 16 compares the HRMS fragmentation of arthrosporol (**64**) and *epi*-arthrosporol (**65**).

Table 16. HRMS for Triol 64 and 65

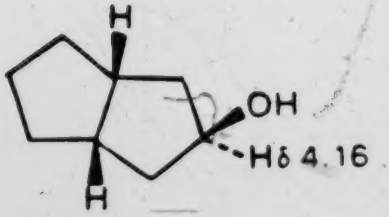
m/z	Fragment	Relative Intensity (%)	
		64	**65**
254	$C_{15}H_{26}O_3$, M^+	absent	33
236	$M^+ - H_2O$	17.8	19.8
218	$M^+ - 2H_2O$	100.0	7.5
203	$C_{14}H_{19}O$	24.6	6.4
182	$C_{11}H_{18}O_2$	32.7	100.0

Table 17. ^1H NMR Spectral Data of Triols 64 and 65

	δ, multiplicity, (J in Hz)	
	64	**65**
H-1	2.38, brdd (13, 8)	2.31, td (11, 1.5)
H-3	2.01, m	2.07, qd (7.2, 5)
H-4	3.95, td (8, 6)	4.14, td (5, 2)
H-5a	2.02, m	2.51,dd (14.1, 5)
H-5b		1.85, dd (14.1, 1.8)
H-14	0.77, s	0.92, s
H-15	1.03, d (7)	1.00, d (7.2)

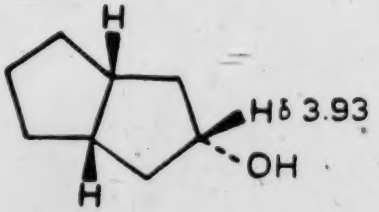
The striking difference between the two spectra is the absence of a molecular ion in the HRMS of triol **64** and the fact that the base peak in **64** corresponds to $C_{13}H_{22}O$, while the base peak in **65** is $C_{11}H_{18}O_2$.

A comparison of the ^1H NMR spectra of the triols (Table 17) reveals a striking difference in the chemical shift and coupling constants of the C-4 carbinyl protons (H-4) as well as in the chemical shift of the angular methyl groups. The chemical shifts of the H-4 methine are similar to the values reported for the C-3 *endo* and *exo*-carbinyl protons in the ^1H NMR spectra of bicyclo[3.3.0]octan-3-ols **69** and **70**.[63]



Endo-H-3 (minor triols)

69



Exo-H-3 (major triols)

70

The chemical shift and coupling constants of H-4 in the ^1H NMR spectrum of triol **65** are similar to the chemical shifts and coupling constants observed in the ^1H NMR spectra of alcohols **47** and **55**. In each of **47** and **55**, the C-4 proton appears as an apparent triplet of doublets (**47** δ 4.31, J = 6 and 2.5 Hz). The coupling constants (J_{cis} = 6 or 5 Hz, J_{trans} = 3 to 2 Hz)[64] observed for the H-4 in minor alcohols **47**, **55**, and **65** are consistent with the stereochemistry proposed for the C-4 hydroxyl group (Figure 8). Consequently the coupling constants observed for the H-4 in ^1H NMR spectra of major alcohols **48**, **52**, **64**, and arthrosporol (**22**) are. J_{trans} = 8 to 9 Hz and J_{cis} = 6 Hz (Figure 9).

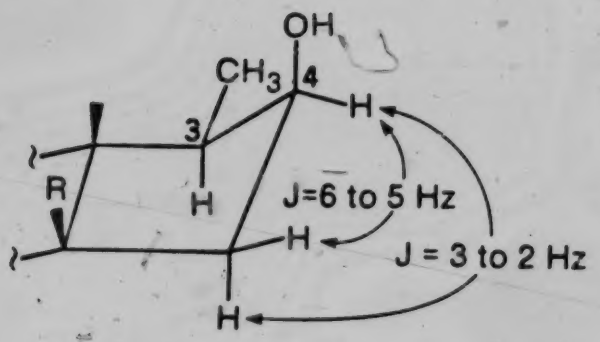


Figure 8

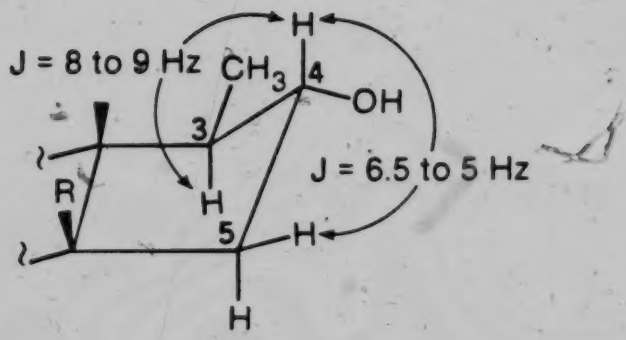


Figure 9

It is interesting to note that when epimeric pairs of alcohols occur, the major alcohols (α) are the most polar. The α-OH is less hindered than the β-OH and thus can hydrogen bond more strongly to the silica gel matrix.

A third alcohol was formed when impure arthrosporone (**20**) was allowed to stir with sodium borohydride in methanol at room temperature. This new compound (m.p. 168-169°, $[\alpha]_D$ -20°C (MeOH)) was assigned structure **71** and named isoarthrosporol on the basis of the evidence which follows.

The molecular formula of **71** was established as $C_{15}H_{24}O_3$ on the basis of a $M^+ - 18$ peak at m/z 236 ($C_{15}H_{24}O_2$). In addition the base peak at m/z 218 ($C_{15}H_{22}O$, 100.0%, $M^+ - 2H_2O$) suggests the presence of at least 2 hydroxyl groups in the molecule. The IR

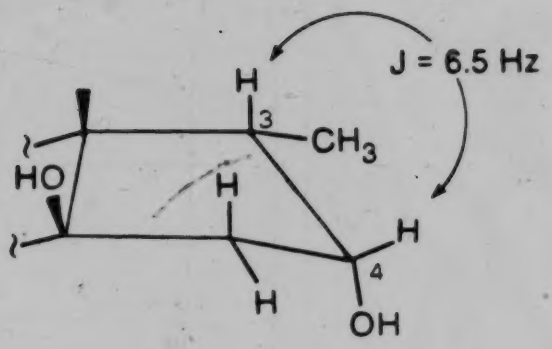
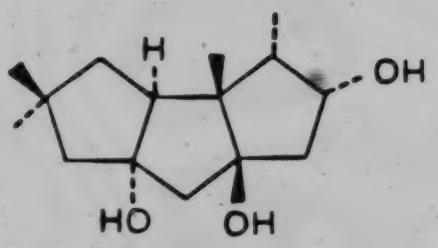


Figure 10

spectrum of **71** shows no carbonyl absorption but does show hydroxyl absorption at 3300 cm^{-1}.

The ^1H NMR spectrum of **71** is similar to the ^1H NMR spectrum of arthrosporol except for the chemical shifts of the C-1, C-3 and C-5 protons (Table 18). The methine proton at C-4 (H-4) in *iso*arthrosporol appears as an apparent broad triplet (J = 6.5 Hz) at δ 4.14 while H-4 proton in arthrosporol appears as an apparent triplet of doublets (J = 9 and 6 Hz) at δ 3.94. The coupling constant J_{cis} = 6.5 Hz for H-4 proton in *iso*arthrosporol (**71**) is consistent with the stereochemical assignment for the C-3 methyl and C-4 hydroxyl groups (Figure 10).

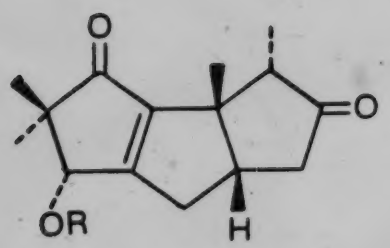


71

Conclusive evidence that *iso*arthrosporol has structure **71** was provided by its successful conversion to isoarthrosporone (**57**).

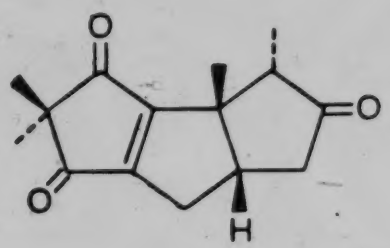
Dehydroarthrosporodione (23)

A fourth compound, which has been assigned structure **23** on the basis of spectroscopic data, was isolated from the Arthrosporae broth extract.



23 R=H

72 R = Ac



73

Compound **23** chars dark purple on TLC using the reagent A visualization technique. Compound **23** is an optically active oil ($[\alpha]_D$ = -80.1°, $CHCl_3$). The HRMS of **23** displays a molecular ion at m/z 248 (M^+, $C_{15}H_{20}O_3$) which is confirmed by CI (m/z 266, M^+ +18). Thus compound **23** has six sites of unsaturation. The base peak at m/z 233 in the HRMS accounts for a loss of a CH_3 fragment while the peak at m/z 230 ($M^+ - H_2O$) indicates the presence of a hydroxyl group in the molecule.

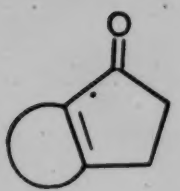
Table 18. ^{1}H NMR Spectral Data for Arthrosporol (22) and Isoarthrosporol (71)

	δ, multiplicity (J in Hz)	
	22	**71**
H-1	2.38, brdd (13, 8)	2.90, ddd (13, 7.5, 1.5)
H-3	2.00, m	1.86, m
H-4	3.94, td (9,6)	4.16, brt (6.5)
H-5a	2.00, m	2.47, brd (16)
H-5b		2.27, brdd (16, 6.5)
H-14	0.77, s	0.89, s
H-15	1.02, d (7)	1.08, d (7)

The IR spectrum of **23** exhibits a strong and broad hydroxyl absorption (3450 cm^{-1}) and strong carbonyl bands at 1738 and 1700 cm^{-1}, along with a C-C double bond absorption at 1637 cm^{-1}.

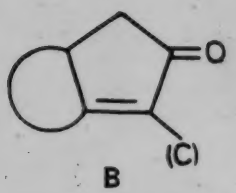
The UV spectrum of the compound shows absorption at λ_{max} 242 nm, suggestive of the presence of chromophores A or B.[44] No shift in absorption was observed when the spectrum of **23** was determined in the presence of acid and base, however, an increase in the intensity of the band (hyperchromic shift) occurred. This indicates that the hydroxyl group observed in the IR and HRMS is not part of the chromophore present in the molecule of **23**.

The ^{13}C NMR spectrum of **23** exhibits four singlet sp^2-hybridized carbon atoms at δ 218.8, 205.8, 185.0, and 145.0 which indicate the presence of a cyclopentanone[33] and an α,β-unsaturated bicyclooctane system C.[41] The peak at 75.1 (doublet) in the ^{13}C NMR spectrum is assigned to the secondary carbinol carbon in the molecule. All three oxygen atoms are thus accounted for. The remainder of the ^{13}C spectrum consists of sp^3-hybridized carbon atoms. They account for two quaternary carbons (singlets), two



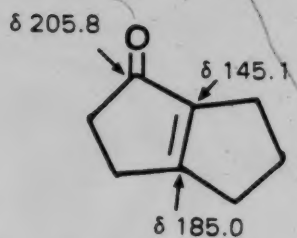
A

λ max (calcd.) = 239 nm



B

λmax (calcd.) = 241 nm

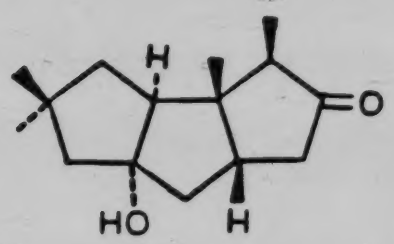


C

methine carbons (doublets), two methylene carbons (triplets), and four methyl carbons (quartets).

The ^1H NMR spectrum of **23** (Figure 11) shows a low field methine proton (H-9) at δ 4.5 (broad dd, J = 6.7 and 2.2 Hz) indicating the presence of an allylic carbinyl proton.[69] On addition of D_2O a signal at δ 1.87 (d, J = 6.7 Hz) disappears and the allylic methine (δ 4.51) collapses to a broad singlet. Decoupling experiments (Scheme XI) show the coupling partners of the allylic methine. The ^1H NMR spectrum confirms the presence of four methyl groups. Three methyl singlets resonate at δ 1.19, 1.18 and 1.09. A methyl doublet (δ 1.17) is coupled to a methine (δ 2.48, q, J = 7 Hz), which has a four-bond coupling with a one-proton multiplet (δ 2.39).

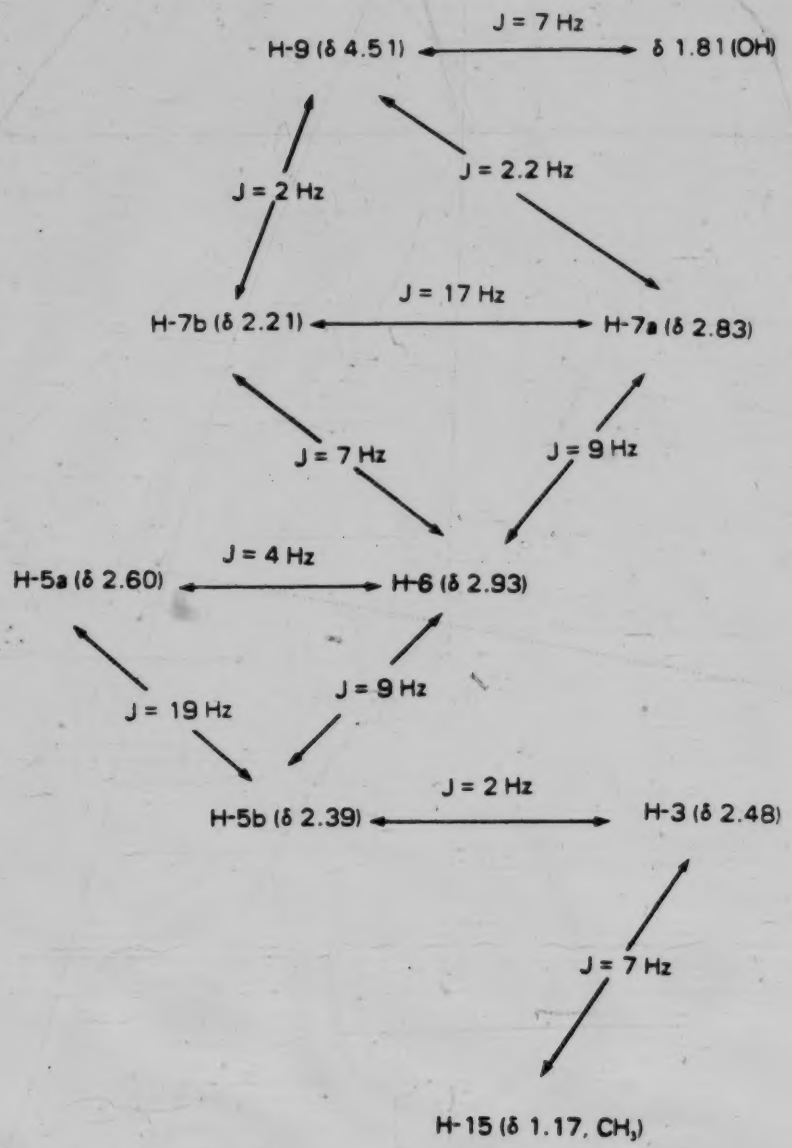
This coupling pattern is analogous to the coupling pattern observed for ring C in the ^1H NMR spectrum of saturated ketone **51** and allows the assignment of those signals to ring C.



51

Evidence for the structural assignment of dehydroarthrosporodione (**23**) was provided by spectral properties of its O-acetyl and triketone derivatives **72** and **73**. Acetylation of compound **23** (Ac_2O, pyridine) gave diketoacetate **72**: (m/z calcd. for $C_{17}H_{22}O_4$: 290.1512; found 290.1524; IR: 1740, 1232 cm^{-1}). Oxidation of **23** (PCC, CH_2Cl_2) yielded triketone **73**: (m/z calcd. for $C_{15}H_{18}O_3$: 246.1251; found: 246.1257; IR: no

Scheme XVI. ^1H NMR Pattern for Dehydroarthrosporodione (23) (400 MHz)



OH, 1696, 1616 cm^{-1}).

The ^1H NMR spectrum of O-acetyldehydroarthrosporodione (**72**) was compared with the ^1H NMR spectrum of dehydroarthrosporodione (**23**). The ^1H NMR spectrum of **72** displays a downfield shift (δ 4.51 in **23** → δ 5.63 in **72**) as expected for the carbinyl proton H-9, which appears as an apparent triplet (J = 2.5 Hz). The C-3 methyl undergoes an upfield shift (δ 1.16 in **23** → δ 1.08 in **72**), implying that the secondary methyl group lies on the same side of the molecule as the acetoxyl group at C-9. Examination of molecular models shows that the methyl group at C-3 should possess an α configuration in order that it be in the anisotropic field of the C-9α acetoxyl group.

Further evidence for the structure of **23** is provided by the ^1H NMR spectrum (see Experimental) of **73**. The ^1H NMR spectrum of **73** shows no carbinyl proton resonance and the long-range coupling of the C-7 methylene protons is not observed.

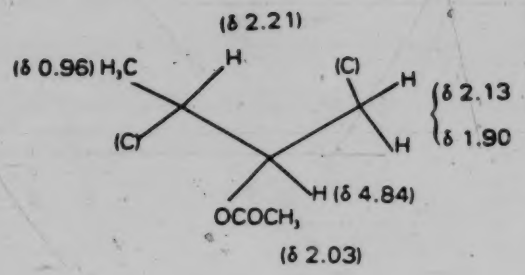
The spectral properties of the derivatives of dehydroarthrosporodione, compounds **72** and **73**, provide support for the assigned substitution pattern in ring A in **23** and the α-configuration of the C-3 methyl group. Lack of material precluded further experiments with this metabolite.

Crude dehydroarthrosporodione (**23**) is usually contaminated with a non UV active compound. Separation of the mixture gave compound **24**, isolated as a white solid (m.p. 150°C with decomposition).

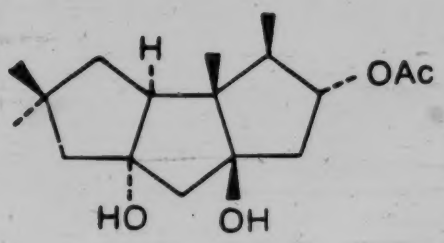
Compound **24** is optically active ($[\alpha]_D$ = -60.9°) and its molecualr weight is 296 as evidenced by CI (m/z 314, M + NH_4^+). The HRMS displays a low intensity peak at m/z 278 ($M^+ - H_2O$). The base peak appears at m/z 218 ($M^+ - HOAc - H_2O$) while a diagnostic peak due to a loss of acetic acid and two molecules of water appears at m/z 200. This information suggested the molecular formula $C_{17}H_{21}O_4$ and four sites of unsaturation for this molecule.

The IR spectrum of the compound shows strong hydroxyl absorption (3540 cm^{-1} (sharp), 3440 cm^{-1} (broad)) and strong bands (1720, 1261 cm^{-1}) indicating the presence of an acetoxyl group in the molecule.

The ^{1}H NMR spectrum of **24** (Figure 12) shows a downfield proton (δ 4.84, apparent dt, J = 4 and 9 Hz), coupled to a methine proton (δ 2.21, J = 9 Hz) and methylene protons (δ 2.13, 1.90, J_{gem} = 14 Hz, J_{cis} = 4 Hz, J_{trans} = 9 Hz). The methine proton at δ 2.21 is coupled to a methyl group at δ 0.96 (J = 7 Hz). A methyl singlet appears at δ 2.03. These data indicate that the partial structure XIX is present in the molecule.



XIX



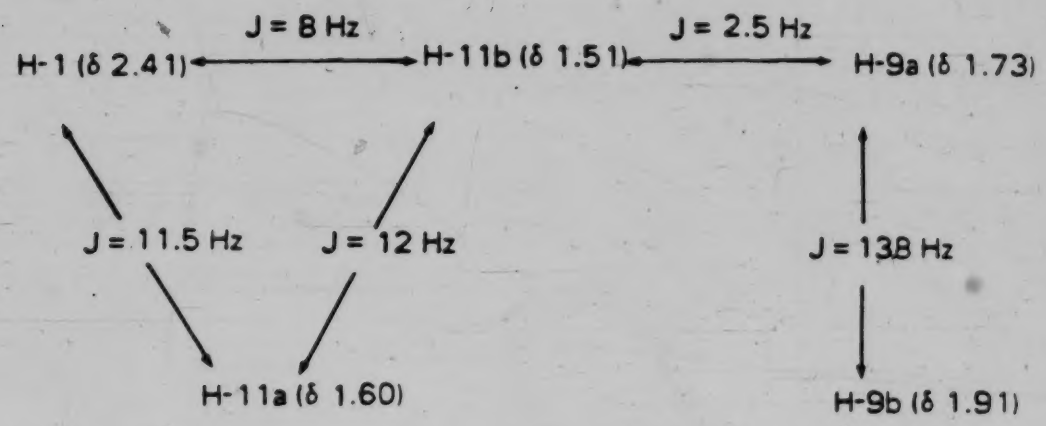
24 ≡ **66**

The ^{1}H NMR spectrum also indicates the presence of an isolated AB spin system (δ 2.27, 2.23, $J_{A,B}$ = 14 Hz), three methyl singlets (δ 1.09, 1.04 and 0.78) and a coupling pattern (Scheme XVII) similar to that previously observed in the ^{1}H NMR spectra of arthrosporone, anhydroarthrosporol and arthrosporol (**20-22**).

From these data it was concluded that compound **24** has the structure shown below. Compound **24** is thus 4-O-acetylarthrosporol. The spectroscopic data of **24** are identical in all aspects with the data obtained for synthetic 4-O-acetylarthrosporol (**66**).

During the course of this work a small amount of a third UV active compound (λ_{max} 229 nm, ϵ 19400) was isolated. This compound was tentatively assigned structure **25** on the basis of the following evidence.

Scheme XVII: ^{1}H Coupling Pattern in 4-O-Acetylarthrosporol (24)



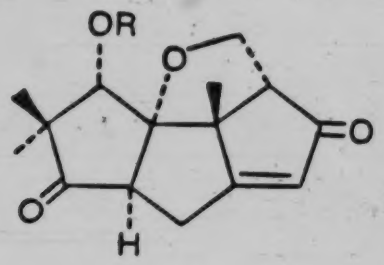
Compound **25** has a specific rotation of +28.9°. ($CHCl_3$). Its IR spectrum displays a hydroxyl absorption at 3456 cm^{-1} (broad) and strong absorption bands (1742, 1704, and 1629 cm^{-1}) characteristic of a cyclopentanone and an α,β-unsaturated cyclopentenone. The molecular weight of 262 (CI m/z 280, 100%, $M+NH_4^+$) and molecular formula $C_{15}H_{18}O_4$ (HRMS m/z 262, 100%, M^+) were determined from the mass spectra of **25**. A fragment ion at m/z 234 ($M^+ - CO$) observed in the HRMS is consistent with the presence of a ketone carbonyl.

The ^{1}H NMR spectrum ($CDCl_3$ + D_2O (1 drop)) of compound **25** (Figure 13) shows the presence of three methyl singlets (δ 1.25 and δ 1.15 ($2\times CH_3$)) and a vinyl proton (δ 5.90 (d, J = 2 Hz)). A one proton singlet at δ 3.92 is consistent with either an epoxide methine[70] or a secondary carbinyl proton. The remainder of the ^{1}H NMR spectrum consists of well resolved spin systems (Scheme XVII) between δ 4.29 and δ 2.76. The lowfield chemical shift of these protons indicates that they are either allylic or *geminal* to an

oxygen atom.

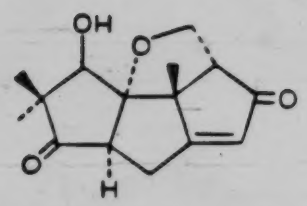
This coupling pattern shows that the vinylic proton (δ 5.9) is allylically coupled to a methylene proton (δ 2.76) which is further coupled to two other protons (δ3.33, 3.07) forming the AB part of an ABX spin system. Decoupling experiments show the presence of another ABX spin system between the protons at δ 4.30, 3.98 and 2.84 (Scheme XVIII).

The data presented for compound **25** to this point is consistent with either structure **74** or structure **75**.

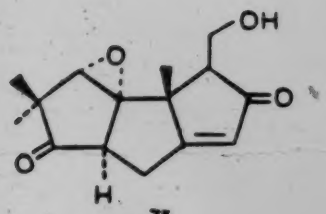


25 R = H

76 R = Ac



74

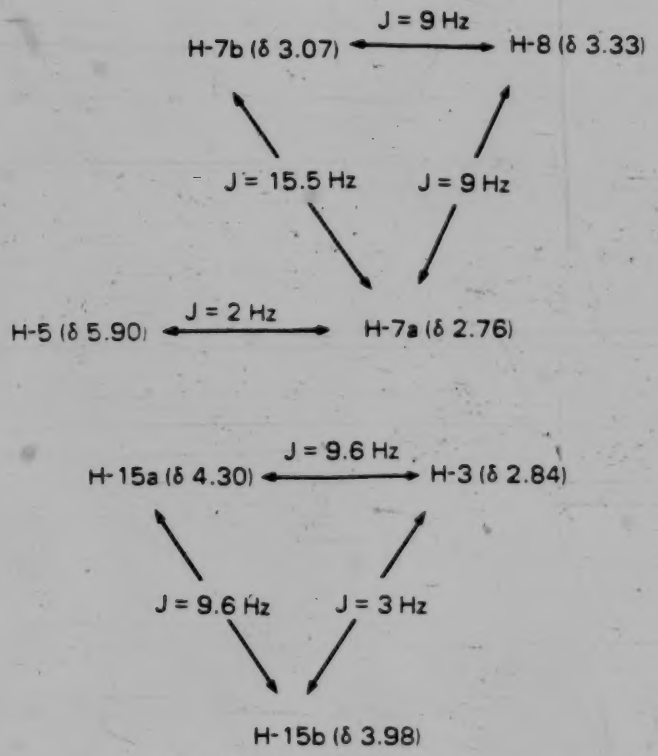


75

When the ^1H NMR spectrum of **25** was obtained in $CDCl_3$ (Figure 14), two doublet protons (δ 3.92 and 2.63, J = 10.5 Hz) were observed. Upon addition of D_2O the signal at δ 2.63 disappeared and the proton at δ 3.92 collapsed to a singlet. Thus these signals indicate the presence of a secondary alcohol and structure **75** may be eliminated from consideration. Further evidence for the tentative structural assignment is based on the spectral properties of the O-acetyl derivative of compound **25**.

Attempted acetylation of **25** with acetic anhydride and pyridine failed. However, acetylation of **25** with acetic anhydride in triethylamine in the presence of

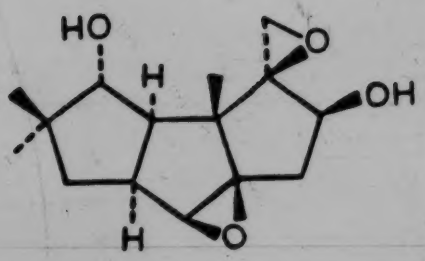
Scheme XVIII: ^1H NMR Coupling Pattern of the Tetracyclic Ether 25 ($CDCl_3$-D_2O)



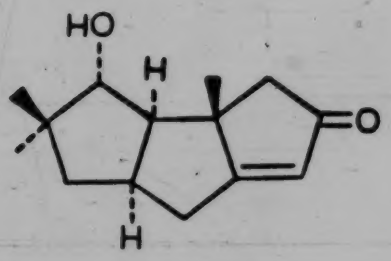
4-dimethylaminopyridine (DMAP) gave a complex mixture from which a UV active acetate **76** was isolated (1744, 1233 cm^{-1} (OAc)).

The ^1H NMR spectrum of acetate **76** shows the expected downfield shift (δ 3.92 → δ 5.09 (s)) for the carbinyl proton at C-11. Two methyl groups in the ^1H NMR spectrum of the acetate experience an anisotropic shift due to the acetoxyl group at C-11 (δ 1.22, 1.15 → δ 1.32, 1.27) and were assigned to the *geminal* methyl groups at C-10. The methine at C-8 experiences a substantial anisotropic shift (Δ = 0.17 ppm) as do the methylene protons at C-15. These observations are consistent with structure **25**.

Further evidence for the configuration of the C-11 hydroxyl group is provided by comparison with the chemical shifts of the carbinyl proton (H-11) in the ^1H NMR spectra of triquinanes **77**[71] and **78**[72] (Table 19).



77



78

The lower field shift observed for the carbinyl proton in **25** is explained by deshielding caused by the C-9 carbonyl group.

Since only very small amounts of compound **25** were obtained, no further experiments were possible with this compound.

Isocyclohumuladiol (26)

Impure fractions of arthrosporone are often contaminated with small amount of a compound, the TLC behavior of which aroused our curiosity. Arthrosporone (**20**) charred green on TLC while the unknown, compound **26**, charred dark blue when they were subjected to the reagent B charring technique. Arthrosporone appeared less polar than the unknown compound when the TLC was run in a solvent system containing acetone as the most polar solvent. On the other hand compound **26** appeared less polar than arthrosporol when the TLC was developed in a solvent system containing methanol as the most polar

Table 19. Chemical Shift for H-11 in 25, 77, and 78

	δ in ppm		
	25	**77**	**78**
H-11	3.92	3.61	3.79

component.

The compound was isolated as a white powder after several chromatographic purifications on silica gel, using chloroform / methanol (96 4), then acetonitrile / dichloromethane (1.3) as solvent systems. The unknown compound is moderately soluble in diethyl ether, chloroform and methanol, and has a specific rotation of +17.5° (MeOH). Attempted recrystallization of **26** (e.g. isopropyl ether / chloroform) produced a powdery material, m.p. = 155°C.

The molecular weight of **26** was determined by HRMS to be 238 ($C_{15}H_{26}O_2$, M^+). In addition to the base peak at m / z 93 (C_7H_9), the HRMS displayed diagnostic peaks at m / z 220 ($M^+ - H_2O$) and 202 ($M^+ - 2H_2O$) indicative of the presence of two hydroxyl groups in the molecule. The formula $C_{15}H_{26}O_2$ requires three sites of unsaturation.

The IR spectrum of **26** displays a strong and broad hydroxyl absorption (3300 cm^{-1}). In addition, a doublet (1382 and 1366 cm^{-1}) suggests the presence of a *gem*-dimethyl group.

Chemical transformations of **26** provides support for the number and the nature of the hydroxyl group(s) in the molecule. Acetylation of **26** (Ac_2O, pyridine) produces a monoacetyl derivative **79** (m / z calcd. for $C_{17}H_{28}O_3$: 280.2038, found: 280.2043, IR 3440, 1731, 1243 cm^{-1}; ^1H NMR δ 2.06 (s, 3H)). Oxidation of **26** (PCC, CH_2Cl_2) gave ketoalcohol **80** (m / z 236 ($C_{15}H_{24}O_2$, M^+), IR 3427 (broad), 1690 cm^{-1}, $[\alpha]_D^{23}$ -117° ($CHCl_3$). These results indicate that compound **26** is a diol with 2° and 3° hydroxyl groups.

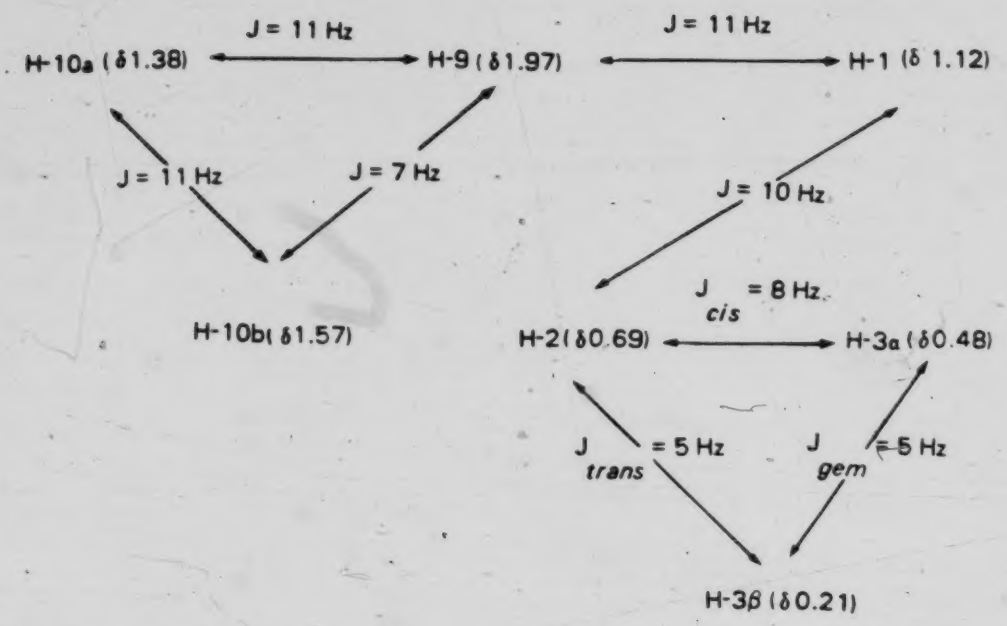
The ^{13}C NMR spectrum of compound **26** shows two carbons bearing oxygen atoms (δ 74.5 (d), 73.6 (s)) consistent with the 2° and 3° nature of the hydroxyl groups in the molecule. Furthermore, the ^{13}C NMR spectrum displays four methyl carbons (quartets), four methylene carbons (triplets), three methine carbons (doublets), and two quaternary carbons (singlets). The most distinctive feature in the ^{13}C NMR spectrum of **26** is the presence of a high-field singlet (δ 20.0) and triplet (δ 18.5). The ^{13}C NMR spectrum confirms the molecular formula of **26** as $C_{15}H_{24}O_2$ by consideration of the multiplicity of the hydrogen-bound carbon atoms. The absence of sp^2 carbon atoms (there are no peaks in the δ 120-200 ppm region), the presence of four methyl groups, and the three unsaturations in the molecule indicate that compound **26** is a tricycloundecanoid

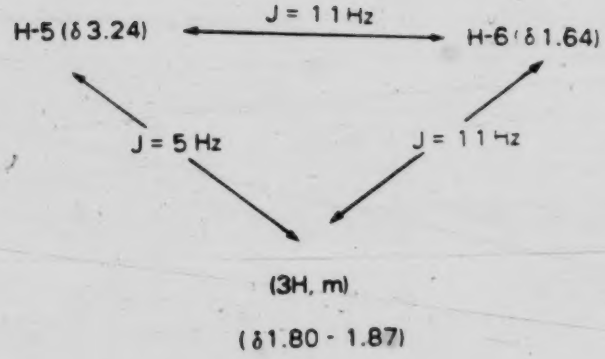
The ^1H NMR spectrum of the tricyclodiol (Figure 15) in $CDCl_3$-D_2O (1 drop), shows the presence of three high field protons (δ 0.69, 0.48, 0.21) which shift to higher field (δ 0.48, 0.23, -0.10) when the solvent is changed from chloroform to benzene. These high-field protons are assigned to a trisubstituted cyclopropane.[73] The presence of a cyclopropane ring accounts for the high-field carbons[74] observed in the ^{13}C NMR spectrum of compound **26**. The ^1H NMR spectrum displays a carbinyl proton signal (δ 3.24) which shifts to δ 2.91 in C_6D_6. The spectrum also confirms the presence of four methyl groups in the molecule (δ 1.15, 1.14, 1.07, 1.04, all singlets).

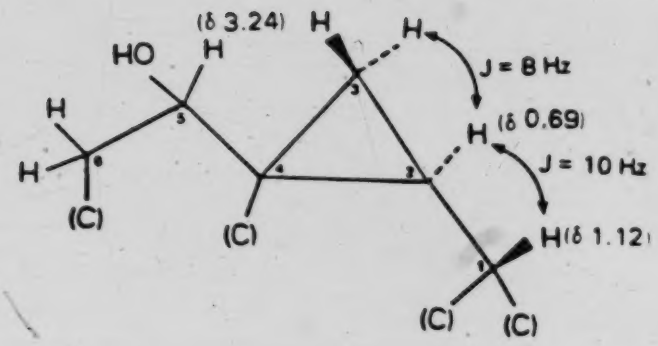
Extensive spin-decoupling experiments (Scheme XIX) allowed the determination of the location and the multiplicity of the remaining protons in the ^1H NMR spectrum of **26**. The carbinyl proton (δ 3.24) appears as a doublet of doublets (J = 11 and 5 Hz). Decoupling experiments reveal the location of only one of its *vicinal* partners. The chemical shift of δ 3.24 is at relatively high field for a carbinyl proton, thus the H-5 proton must be in close proximity to the cyclopropyl ring. This suggests that sequence A is present in the molecule.

Chemical shifts of the protons in the cyclopropane ring were assigned on the basis of the coupling constants of their respective spin systems. H-3β (δ 0.21) appears as an apparent triplet ($J_{gem} = J_{trans}$ = 5 Hz) while H-3α appears as a doublet of doublets (J_{gem} = 5 Hz, J_{cis} = 8 Hz). The chemical shifts and coupling constants of compounds containing partial

Scheme XIX: ^1H NMR Coupling Pattern of Tricyclodiol 26 (400 MHz)





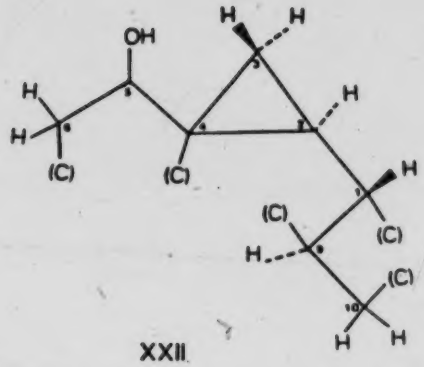


A

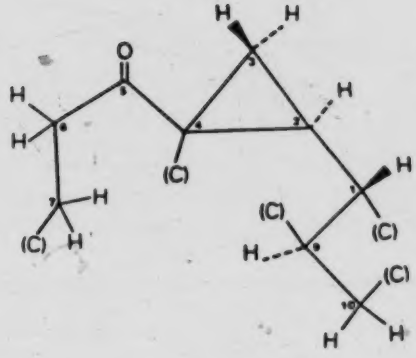
structure A are well documented in the literature.[73] Spin decoupling experiments (Scheme XIX) show that H-1 (δ 1.12) appears as an apparent triplet (J = 10 Hz). The J value is large enough to assume a *trans* relationship between the C-1 and C-9 protons.[74] H-9 (δ 1.97) is also coupled to the C-10 methylenes (δ 1.57, 1.39. J_{cis} = 7 Hz, J_{trans} = 11 Hz) which have a 11 Hz *geminal* coupling. These data are consistent with the partial structure XXII.

Examination of the spectral properties of the derived ketoalcohol **80** gave further information about the structure of **26**. The spectral data for ketoalcohol **80** indicates the presence of the partial structure XXIII. The presence of a cyclopropyl ketone is consistent with the IR spectrum ($\nu_{C=O}$ = 1698 cm^{-1}).[75]

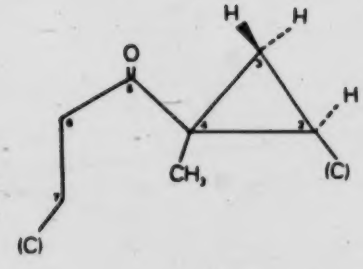
The ^1H NMR spectrum of **80** shows signals which can be attributed to H-2 (δ 0.95 m) and the C-3 methylene protons (δ 1.19 (t, J = 5.5 Hz); δ 0.52 (dd, J = 8 and 5.5 Hz)). H-2 and H-3β are deshielded with respect to their chemical shifts in the ^1H NMR spectrum of diol **26**. The ^1H NMR spectrum of ketoalcohol **80** also shows a deshielding of one of the methyl singlets which appears at δ 1.38. This methyl is *vicinal* to the C=O or *geminal* to a hydroxyl group (partial structure XXIV or XXV). The C-6α proton (δ 2.42) appears as an apparent triplet of doublets (J = 13.5 and 4 Hz). H-7α (δ 2.34) has an identical spin system



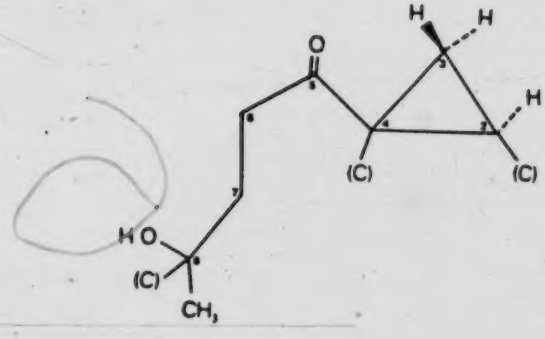
XXII



XXIII

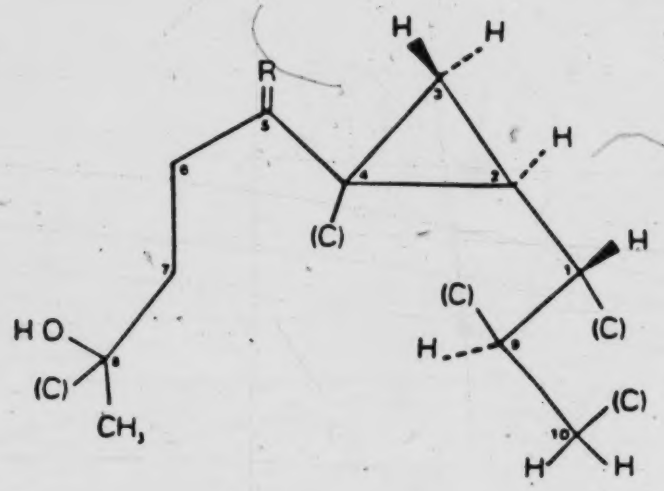


XXIV



XXV

as that of H-6α. H-6β (δ 2.79) and H-7β(δ 1.93)have the same spin systems (triple doublet (ddd). J = 13.5, 5.1 and 3.5 Hz). The chemical shift assignments of the C-6 and C-7 methylene protons are confirmed by spin decoupling experiments and are consistent with values reported for similar systems found in the literature.[71] Chemical shifts for the C-4 methyl group in cyclopropylketones analogous to XXIV are between δ 1.15 and δ 1.20 ppm.[71] The ^{1}H NMR spectrum of **80** displays four methyl singlets at δ 1.36, 1.19, 1.06 and 0.96. Chemical shifts (δ 1.36 and 1.19) account for the methyl singlets at C-8 and C-4 in partial structure XXVI. Thus partial structure XXVII is present in the structure of diol **26**.



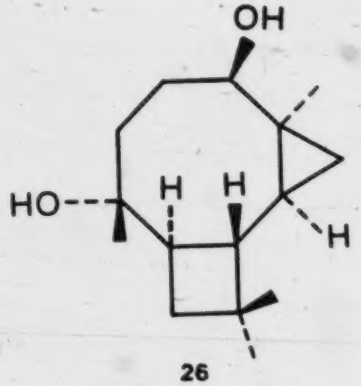
XXVI R = O

XXVII R =

The ^{13}C NMR spectrum of the diol displays only three quarternary carbons and partial structure XXVII accounts for two of these. Since there are four methyl singlets in the structure of the diol, one of the quaternary carbons must bear two methyl groups. The *gem* dimethyl group cannot be *geminal* to the C-8 OH, since an open chain will result and there are not sufficient carbons left to form these rings. Insertion of the carbon bearing 2 methyl groups between C-1 and C-10, and connecting C-8 to C-9 gives structure **26** for the diol.

Table 20. ^1H NMR Spectra Data for Isocyclohumuladiol (26)

	δ in ppm		
	$CDCl_3$	C_5D_5N	Δ
H-12	1.05	1.03	0.02
H-13	1.07	1.13	-0.06
H-14	1.15	1.16	+0.01
H-15	1.14	1.28	-0.14

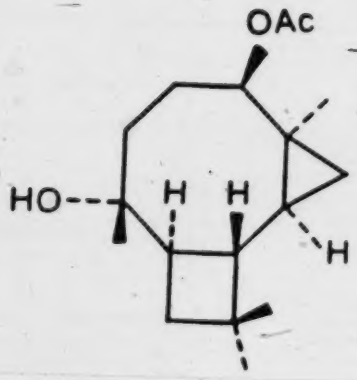


26

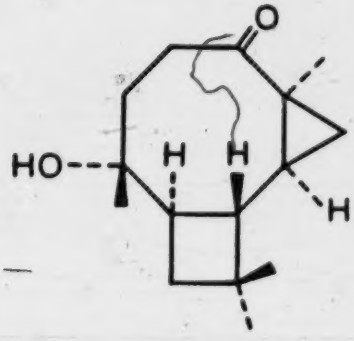
The stereochemistry at C-1, C-2 and C-9 was deduced from the coupling constants of H-1, H-2, and H-9. The relative stereochemistry at C-4 was assigned on the basis of the following observations. The ^{1}H NMR of **26** in pyridine shows a pyridine induced shift for only one methyl group (Table 20).

The C-8 methyl group experiences a strong deshielding in the ^{1}H NMR spectrum of ketoalcohol **80** (Δ = 0.24 ppm) relative to the ^{1}H NMR spectrum of diol **26**. The protons at C-1, C-2, and C-3 also experience significant deshielding by the ketone indicating that they are near the carbonyl group.

The *trans* stereochemistry between the methyl at C-4 and the hydroxyl group at C-5 is supported by the fact that the methyl group does not undergo an acetoxyl anisotropic shift in the ^{1}H NMR spectrum of the acetoxyalcohol **79**.



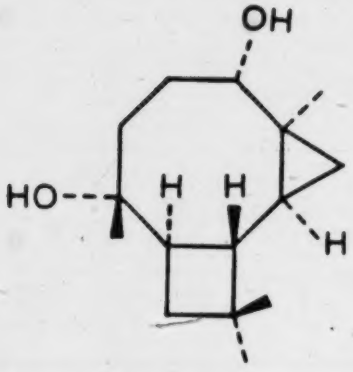
79



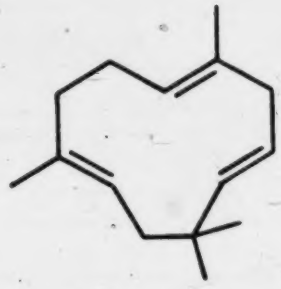
80

A search of the literature reveals that the epimeric alcohols **26** and **81** are known compounds. Compound **81**, named tricyclohumuladiol, has been reported as a constituent of hop-oil[86] and has also been prepared by cyclization of humulene (**82**)[81, 83] Compound **26** was prepared by sodium borohydride reduction of the ketone (**80**) obtained by oxidation of **81**.

Tricyclohumuladiol (81) has a melting point of 205-207°C and that isolated from hop oil is reported as having $[\alpha]_D = 0°$. The compounds prepared from humulene are necessarily racemic. The melting point of our compound (26) is 155°C and it is dextrorotatory. The melting point of racemic 26 is reported as 106°C. The spectral data given in the literature is very incomplete.[80-82] For example the splitting of the carbinyl proton in 81 is given as 18 and 22 Hz (obviously incorrect) and the chemical shift is not reported. The shift of the H-5 proton in 81 is recorded at δ 2.98 (multiplet) in one paper [80] and at δ 3.30 in the other. The ketonic carbonyl absorption of 80 is reported as 1670 cm^{-1} in one paper 1685 (1690) cm^{-1} in others. The only information recorded for 26 is the melting point and the splitting of the C-5 carbinyl proton (triplet, J = 9 Hz). Because of these discrepancies and the fact that we had insufficient material for further studies, we are attempting to obtain authentic samples of 26 and 81. Our structural assignment must remain uncertain until direct comparisons are made.*



81



82

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*Comparison with an authentic sample of racemic 81 shows that the metabolite 26 is in fact one enantiomer of compound 81.

III. EXPERIMENTAL

Distilled water used to prepare growth media was redistilled using an all glass distillation apparatus. DIFCO bacto potato dextrose broth (PDB) or potato dextrose agar (PDA) and yeast extract were utilized for growth media. New Brunswick Scientific MF-214 microferm and magnaferm laboratory ten-liter fermentors were used for large fermentation. Still culture fermentation was performed using 2.5-liter Fernbach flasks. Celite 545 (American Chemicals Limited) was used as a filter aid. Reagent grade solvents were distilled prior to use. Skellysolve B refers to Skelly Oil Company light petroleum, b.p. 62-70°C. Analytical grade diethyl ether (ACS 288) from freshly opened cans was used without further purification. Column chromatography was carried out using Brinkman Instrument silica gel 60 (minus 0.080 mm / 200 mesh). E. Merck silica gel 60 (0.040 - 0.063 mm / 230-400mesh) was used for flash column chromatography. Analytical thin layer chromatography was carried out on home-made plates prepared using thin layer silica gel G (Terochem) containing 1% of Retma P-1 electronic phosphor (General Electric), or on aluminum foil supported silica gel 60 (E. Merck, 0.25 mm, F_{254}) plates. The chromatograms were examined under ultraviolet light (λ = 254 nm or λ = 350 nm). The developed plates were visualized by spraying with aqueous 20% sulfuric acid containing 1% vanillin (reagent A), or dipping in 5% phosphomolybdic acid in aqueous 5% sulfuric acid containing a trace of ceric sulfate (reagent B). The stained plates were charred by slowly heating them on a hot plate.

Melting points were taken on a Fisher-Johns melting point apparatus and are uncorrected. Fourier transform infrared (FTIR) spectra were obtained on a Nicolet 7199 F.T. interferometer. Ultraviolet (UV) spectra were recorded on a UNICAM SP 1700 Ultraviolet spectrophotometer, or on a Hewlett-Packard HP8450A Diode Array spectrometer coupled to a 7470A plotter. Optical rotations (OR) were determined on a Perkin-Elmer model 141 automatic polarimeter. High resolution electron impact mass spectra (HREIMS) were recorded on an AEI MS-50 mass spectrometer coupled to a data processing DS-50 computer system. Chemical ionization mass spectra (CIMS) and low resolution electron impact mass spectra (LREIMS) were determined on an AEI MS-4 mass

spectrometer coupled to a DS-9 computer system. Data are reported as m/z (relative intensity). High field proton nuclear magnetic resonance (^{1}H NMR) and carbon nuclear magnetic resonance (^{13}C NMR) spectra were recorded on a Brucker WH-400 spectrometer coupled to an Aspect 2000 computer system. Chemical shifts expressed in δ units are reported in parts per million (ppm) downfield from tetramethylsilane (TMS), the internal standard. Coupling constants, J, are expressed in cycles per second (Hertz, Hz) and the following abbreviations are used: m = multiplet, s = singlet, d = doublet, t = triplet, q = quartet, br = broad.

Growth of the Fungus

An Arthrosporae fungus, discovered by Tsuneda and Hiratsuka[1] has been deposited at the University of Alberta Mold Herbarium under the accession number UAMH 4262.* The fungus, believed to be a haploid Basidiomycetes, has not yet been fully identified.

The liquid growth medium (PDBY) was obtained by adding 2 g of yeast extract to a 1 L solution of potato dextrose broth (Difco). The solid medium (PDAY) was prepared from PDBY by addition of agar (20 g/L). The media were autoclaved at 121°C for 20 to 45 min depending on the volume of medium to be sterilized.

Arthrosporae (UAMH 4262) was maintained at 4°C in slant tubes containing PDAY. These slant tube cultures were used as maintenance stock cultures. Inoculum ofArthrosporae was prepared by adding 2 mL doubly distilled, sterile water to one maintenance culture. The surface of the agar was scratched lightly with a sterile needle to produce a mycelial suspension. The suspension was used to inoculate two petri dish plates containing PDAY. The inoculated plates were maintained at 15 to 20°C for two weeks. A mycelial suspension (5 mL), prepared as previously described from one plate, was transferred to a 500 mL Erlenmeyer flask containing 200 mL of sterilized liquid medium (PDBY). Two inoculated flasks were maintained at 15 to 20°C on a gyrotory shaker for two

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* Identification code of the Arthrosporae fungus deposited at the University of Alberta Mold Herbarium under the accession number UAMH 4262.

weeks. The shake culture (400 mL) was transferred aseptically to a fermentor containing 10 L of sterilized PDBY and antifoaming agent (polypropylene glycol, 1 mL).* The fermentation culture was maintained at 18°C for four weeks (agitation rate = 200 rpm).

Still cultures were prepared by transferring 25 mL of inoculum, prepared as described above, into a Fernbach flask containing 1 L of sterilized PDBY. The cultures were allowed to stand at 15 to 20°C for one month.

Extraction of the Crude Metabolites

Regardless of the fermentation method used, metabolites were isolated in the following manner: The mycelia were separated from the broth by filtration through glass wool using a Buchner funnel. The mycelia were allowed to air dry in a fume-hood for ten days, then were extracted as described below. Celite was added to the broth (5 g / L) and mixed to give a homogeneous suspension. The suspension was filtered *in vacuo* (Whatman filter paper number two) to give a clear, mycelium-free filtrate. The broth filtrate (10 L from still cultures) was concentrated *in vacuo* at 55°C (water bath) to give 2 L of concentrate. The concentrate was continuously extracted for two days in a liquid-liquid extractor first with diethyl ether (ether), then with ethyl acetate. The ether solution was dried over anhydrous magnesium sulfate ($MgSO_4$) and concentrated to dryness under reduced pressure to give the ether soluble extract, E_1 (1.058 g). The ethyl acetate extract was dried and concentrated in a similar manner to yield the ethyl acetate soluble extract, E_2 (0.398 g).

The air-dried mycelia from still cultures were pulverized and extracted for three days in a Soxhlet extractor with ether, then with ethyl acetate. The organic extract was washed (water and brine), dried over sodium sulfate (Na_2SO_4) and concentrated to dryness *in vacuo* to give the mycelium extract E_M-1 (1.480 g) from ether and the mycelium extract E_M-2 (2.408 g) from ethyl acetate.

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*The antifoaming agent is omitted when the fermentor possesses a foam-breaker.

Separation of the Neutral Metabolites

The ether soluble extracts, E_1 (2.145 g), were dissolved in ethyl acetate (500 mL) and the solution was extracted with 5% aqueous sodium bicarbonate (2x50 mL). The organic layer was washed with water (50 mL) and further extracted with 5% aqueous sodium hydroxide solution (2x50 mL). The ethyl acetate solution was washed with water (50 mL) and brine (50 mL), then dried ($MgSO_4$). The solvent was removed at 30°C under reduced pressure to give a strong smelling extract, E_N (0.969 g). Each of the basic aqueous extracts were worked up as follows: The aqueous extract was acidified and extracted with ethyl acetate. The organic extract was dried ($MgSO_4$) and concentrated *in vacuo*. In this manner an extract containing mainly carboxylic acids, E_A-1 (0.150 g) and an extract containing mainly phenolic compounds, E_A-2 (0.388 g), was obtained.

Preliminary Purification

A 50 mm (outer diameter) column was packed with silica gel (6.5 in) for flash chromatography using dichloromethane. The neutral crude extract (1.430 g) was dissolved in dichloromethane (5 mL) and applied to the column through a cotton plug. The column was eluted at a flow rate of 2 inches per minute using gradient solvent elution with acetonitrile (Table 1). The volume of aliquot was generally 40 mL but 25 mL aliquots were collected during the elution of the compounds of interest. The fractions were monitored by TLC using an acetonitrile / dichloromethane (1:1) solvent system.* UV was used for detection or plates were visualized using the reagent / char technique. Several fractions were collected with each solvent mixture. System A was used to remove greasy or very low polarity material. System B usually eluted the phthalates along with a complex tailing material. System C and D eluted the C_{13} metabolites, fractionated as shown in Table 2.

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*TLC plates were sprayed with 1% vanillin in 20% or 15% sulfuric acid, followed by careful heating using a hot plate.

Table 1. Polarity of the Gradient Elution

Solvent	System	Ratio	Volume
A	CH_2Cl_2	–	500
	CH_2Cl_2-CH_3CN	14:1	500
B	CH_2Cl_2-CH_3CN	7:1	300
	CH_2Cl_2-CH_3CN	7:2	500
C	CH_2Cl_2-CH_3CN	3:2	3100
D	CH_2Cl_2-CH_3CN	1:1	1000
E	Me_2CO-MeOH	–	1000

Table 2. Acetonitrile dichloromethane (2:3) fractions

Fraction	Yield (mg)	Observations
80-99	29.5	mixture **23** and **24**
100-118	53.0	
119-122	16.8	mixture **20**, **21** and **23**
123-150	146.6	mixture **20**, **21** and **25**
151-160	19.8	mixture **20**, **21** and **26**
190-217	49.7	crude **22**

Isolation of arthrosporone (20), tetracyclic ether (25) and *isocyclohumuladiol* (26)

An impure fraction (110 mg) containing **20** was chromatographed over silica gel (10 g) by elution with 10% acetone in dichloromethane. Fractions (3 mL) were collected using an automatic collector. The fractions were monitored by TLC (acetone / dichloromethane 3:7) and were combined to ten final fractions (A-K). Fractions C (4 mg) and K (55 mg) were subjected to further purification.

***cis*-2β,10,10-Trimethyl-11α-hydroxy-(1,3) epoxymethanotricyclo [6.3.0.0^{2,6}] undec-5-en-4-one (tetracyclic ether, 25)**

A mini column was prepared by packing a disposable Pasteur pipette (5 3/4 in, Fisher Scientific Ltd.) with a slurry of silica gel in ethyl acetate / Skellysolve B (5:95). Fraction C (3.5 mg) was applied to the column and the column was eluted with ethyl acetate / Skellysolve B (3:7). Fractions (0.4 mL) were collected and monitored by TLC (EtOAc / SkB 1:1). Compound **25** eluted in vials 10 to 13. Fraction 11 yielded a pure UV active compound (1.7 mg): m.p. (not available).

TLC: R_f * 0.23 (ethyl acetate / pentane 2:3), yellow-greenish spot (reagent A).

OR: $[\alpha]_D^{23}$ + 28.9° (*c* .92, $CHCl_3$)

UV (MeOH): λ_{max} 229 nm (ε 19400)

FTIR (cast): 3456 (broad) 3070, 1740 (strong), 1704 (strong), 1629 (strong), 1080 cm^{-1}.

HREIMS: m/z (formula, intensity, fragment) 262 ($C_{15}H_{18}O_4$, 100.0, $M^{+\cdot}$), 234 ($C_{14}H_{18}O_3$, 3.5, $M^{+\cdot} - CO$), 191 ($C_{11}H_{11}O_3$, 36.5, $M^{+\cdot} - C_4H_7O$), 163 ($C_{10}H_{11}O_2$, 11.7, $M^{+\cdot} - C_5H_7O_2$), 72 (C_4H_8O, 3.2, $M^{+\cdot} - C_{11}H_{10}O_3$).

CIMS: m/z (intensity, fragment) 280 (100.0, $M{\cdot}NH_4^+$), 263 (43.2 ($M{\cdot}H^+$), 262 (21.0, $M^{+\cdot}$).

^{1}H NMR (400 MHz, $CDCl_3$-D_2O): δ 5.90 (1H, d, 2.0 Hz, H-5) 4.29 (1H, t, 9.6 Hz, H-15a), 3.98 (1H, dd, 3.0 and 9.3 Hz, H-15b), 3.92 (1H, s, H-11),** 3.33 (1H, t, 9.0 Hz, H-8), 3.07 (1H, dd, 9.0 and 15.5 Hz, H-7a), 2.84 (1H, dd, 3.0 and 9.5 Hz, H-3), 2.76 (1H, ddd, 2.2, 9.0 and 16.0 Hz, H-7b), 1.25 (3H, s, C-10 Me) 1.15 (6H, s, C-10 Me and C-3 Me).

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*Unless otherwise, the R_F of the compounds are given using cholesterol as reference.

**δ ($CDCl_3$) 3.93 (d, J = 10.5 Hz) *vicinal* to a hydroxy proton at δ 2.63 (d, J = 10.5 Hz, D_2O exchangeable).

Purification of *cis,anti,cis*-2β,3β,10,10 -tetramethyl-6β,8α-dihydroxytricyclo [6.3.0.0^{2,6}] undecan-4-one (arthrosporone, 20) and the minor compound 26

Fraction K (21.2 mg) previously obtained was chromatographed on silica gel (flash chromatography) using acetonitrile / dichloromethane (1 3) as eluant. Nine fractions were collected (F-1 to F-9) and the fractions F-3 and F-4 (8.9 mg) contained a single compound. Recrystallization from Skellysolve B / diethyl ether yielded analytically pure arthrosporone **20** as colorless crystalline needles, m.p. 139-141°C.

TLC R_f 0.50 (acetone / benzene 3 2) reddish spot turning quickly to a greyish blue (reagent A).

OR $[\alpha]_D^{23}$ -140.8° (*c* .9, $CHCl_3$).

UV (MeOH) λ_{max} 280 nm (ε 650).

FTIR ($CHCl_3$, cast) 3440 (br), 2951 (s), 2866, 1731 (strong), 1381, 1360, 1275, 1189, 1015 cm^{-1}.

HREIMS m / z (formula, intensity, fragment) 252.1722 ($C_{15}H_{24}O_3$, 19.4, M^+), 234 ($C_{15}H_{22}O_2$, 27.8, $M^+ - H_2O$), 219 ($C_{14}H_{20}O_2$, 13.4, $M^+ - H_2O-CH_3$), 216 ($C_{15}H_{20}O$, 2.0, $M^+ - 2H_2O$), 206 ($C_{14}H_{22}O$, 12.4, $M^+ - H_2O-CO$), 192 ($C_{13}H_{20}O$, 89.4, $M^+ - C_2H_2O-H_2O$), 191 ($C_{13}H_{19}O$, 35.4, $M^+ - C_2H_2O-H_2O$), 177 ($C_{12}H_{17}O$, 22.4, $M^+ - C_3H_7O$), 163 ($C_{11}H_{15}O$, 20.6, $M^+ - C_4H_9O_2$), 127 ($C_7H_{11}O_2$, 2.3, $M^+ - C_8H_{13}O$), 125 ($C_8H_{13}O$, 2.7, $M^+ - C_7H_{11}O_2$), 125 ($C_7H_9O_2$, 100.0, $M^+ - C_8H_{15}O$), 109 (C_8H_{13}, 25.1, $M^+ - C_7H_{11}O_3$), 95 (C_7H_{11}, 32.9, $M^+ - C_8H_{13}O_3$), 83 (C_5H_7O, 39.6, $M^+ - C_{10}H_{17}O_2$) 69 (C_5H_9, 18.0, $M^+ - C_{10}H_{15}O_3$), 56 (C_4H_7, 13.1, $M^+ - C_{11}H_{17}O_3$).

CIMS m / z (intensity, fragment) 270 (100.0, $M \cdot NH_4^+$) 252 (39.6 M^+), 235 (38.0 $MH^+ - H_2O$).

^{1}H NMR (400 MHz, $CDCl_3$) δ 2.69 (1H, dd, 19.8 and 1 Hz, H-5a) 2.57 (1H, brq, 7 Hz, H-3), 2.56 (1H, dd, 12 and 8 Hz, H-1), 2.40 (1H, brd, 16 Hz, H-7a), 2.21 (1H, d, 16 Hz, H-7b), 2.20(1H, d, 19.5 Hz,H-5b), 1.96 (1H, brd, 14 Hz, H-9a), 1.79 (1H, dd, 14 and 2.5 Hz, H-9b), 1.70 (1H, brt, 12 Hz, H-11a), 1.59 (1H, ddd, 12, 8 and 2.5 Hz, H-11b), 1.16 (3H, s, H-12) 1.08 (3H, s, H-13), 1.02 (3H, d, 7 Hz, H-15), 0.84 (3H, s, H-14).

^{1}H NMR (C_5D_5N) δ 5.60 (1H, s, OH), 2.92 (1H, qd, 7 and 1 Hz, H-3), 2.77 (1H, dd, 12 and 9.5 Hz, H-1), 2.75 (1H, d, 16 Hz, H-7a), 2.55 (1H, d, 20 Hz, H-5a), 2.47 (1H, dd, 20 and 1 Hz, H-5b), 2.46 (1H, d, 16 Hz, H-7b), 2.14 (1H, d, 14 Hz, H-9a), 2.06 (1H, dd, 14 and 3 Hz,

H-9b), 1.97 (1H, brt, 12 Hz, H-11a), 1.47 (1H, ddd, 12, 9.5 and 3 Hz, H-11b), 1.24 (3H, s, H-12), 1.00 (3H, d, 7 Hz, H-15) 0.96 (3H, s, H-13), 0.94 (3H, s, H-14).

^{13}C NMR (100 MHz, $CDCl_3$) δ 216.4 (weak, s, C-4), 91 (s, C-6) 87.0 (s, C-8), 60.6 (d, C-3), 58.9 (t, C-5), 56.9 (t, C-7) 55.5 (d, C-1), 54.6 (s, C-2), 49.9 (t, cC-9), 44.7 (t, C-11) 40.2 (s, C-10), 29.6 (q), 26.9 (q), 11.3 (q), 8.3 (q).

***trans,cis*-4α-8β,11,11-Tetramethyltricyclo [7.2.0.0^{2,4}] undecan-5β,8α-diol (*iso*cyclohumuladiol, 26)**

Fraction 7 (1.5 mg) contained a single minor metabolite which was identified as compound **26**. All attempts to recrystallize **26** produced only powdered material, m.p. 155°C.

TLC R_f 0.48 (acetone / benzene 2.3), 0.35 (ethyl acetate / pentane 1.2, developmentx2). Produced dark blue spot with reagent B / char technique.*

OR $[\alpha_D]^{23}$ +17.5 (*c*, 1.3, MeOH).

FTIR ($CHCl_3$, cast): 3400-3200 (broad), 3056 (weak) 1453, 1452, 1382 and 1366 (as a doublet), 1073, 1011 and 894 cm^{-1}.

HREIMS m / z (formula, intensity) 238.1931 ($C_{15}H_{26}O_2$, 2.5, $M^{\cdot}$), ($C_{15}H_{24}O$, 5.4, $M^{\cdot} - H_2O$), 205 ($C_{14}H_{21}O$, 6.0, $M^{\cdot} - H\,CH_3O$) 202 ($C_{15}H_{22}$, 1.4, $M^{\cdot} - 2H_2O$), 164 ($C_{11}H_{16}O$, 16.7, $M^{\cdot} - C_4H_{10}O$), 162 ($C_{12}H_{18}$, 21.0), 147 ($C_{11}H_{15}$, 23.7), 138 ($C_9H_{14}O$, 21.2), 121 (C_9H_{13}, 50.4), 111 ($C_7H_{11}O$, 27.0, $M^{\cdot} - C_8H_{15}O$), 109 (C_8H_{13}, 33.5, C_7H_9O, 38.6), 97 (C_6H_9O, 41.1), 95 (C_7H_{11}, 89.4 and C_6H_7O, 18.1), 94 (C_7H_{10}, 54.1), 93 (C_7H_9, 100.0), 84 (C_5H_9O, 50.3), 81 (C_6H_9, 71.3), 79 (C_6H_7, 58.0), 67 (C_4H_7O, 67.1), 59 C_3H_7O, 59.8) and 55 (C_4H_7, 92.6).

CIMS m / z (intensity, fragment) 256 (0.1, $M{\cdot}NH_4^{\cdot}$), 238 (32.8, $M{\cdot}NH_4^{\cdot}$-H_2O), 221 (100.0, $MH^{\cdot}$-H_2O), 220 (2.6, $M{\cdot}NH_4^{\cdot}$-$2H_2O$).

^{1}H NMR (400 MHz, $CDCl_3$-D_2O) δ 3.24 (1H, dd, 5.0 and 11.0 Hz, H-5), 1.97 (1H, td, 11.0 and 8.0, H-9), 1.87 (1H, m), 1.84 to 1.80 (2H, m), 1.64 (1H, t, 11.0), 1.57 (1H, dd, 8.0 and 11.0 Hz, H-10a) 1.38 (1H, t, 11.0 Hz, H-10b), 1.15 (3H, s, H-14), 1.14 (3H, s, H-15), 1.12 (1H, t, 11 Hz, H-1), 1.07 (3H, s, H-13 or H-14) 1.05 (3H, s, H-14 or H-13), 0.69 (1H,

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*The major metabolite (1 produced greenish spot in the same condition.

ddd, 10.5, 8 and 5 Hz, H-2), 0.48 (1H, dd, 8 and 5 Hz, H-3α),* 0.23 (1H, t, 5.0Hz, H-3β).

^{1}H NMR (C_6D_6): δ 2.91 (1H, dd, 11 and 5 Hz, H-5), 1.90(1H, td, 11 and 7 Hz, H-9), 1.73 (1H, dd, 12 and 5 Hz, H-6a), 1.68-1.63 (2H, m, H-6 and H-7), 1.57 (1H, t, 11 Hz, H-7), 1.47 (1H, dd, 11 and 8 Hz, H-10a), 1.28 (1H, t, 11 Hz, H-10b), 1.04 (3H, s, H-12 or H-13), 0.99 (3H, s, H-13 or H-12), 0.92 (3H, s, H-14 or H-15), 0.90 (3H, s, H-15 or H-14) 0.75 (1H, t, 11 Hz, H-1), 0.48 (1H, ddd, 11, 7 and 5 Hz, H-2), 0.23 (1H, dd, 7 and 5 Hz, H-3α), -0.10 (1H, t, 5 Hz, H-3β).

^{1}H NMR (C_5D_5N): δ 3.50(1H, m, H-5), 2.21 (1H, brq, 10 Hz, H-9), 2.06 (3H, m), 1.88(1H, brt, 11 Hz, H-6), 1.70(1H, dd, 9.5 and Hz, H-10a), 1.51 (t, 10.5, H-10b), 1.28 (3H, s, H-15), 1.23 (1H, t, 10.5 Hz, H-1), 1.16 (3H, s, H-14), 1.13 (3H, s, H-12 or H-13) 1.03 (3H, s, H-13 or H-12), 0.70(1H, ddd, 10.5, 8 and 5 Hz, H-2) (1H, dd, 8 and 4.6 Hz, H-3α), 0.36 (1H, t, 48 Hz, H-3β).

^{13}C NMR (100MHz, CD_3OD): δ74.5 (d, C-5), 7.36 (s, C-8), 58.7 (s, C-11), 49.1 (d, C-9), 48.3 (d, C-1), 40.0 (t, C-6 or C-7), 33.7 (t, C-7 or C-6), 30.5 (d, C-2) 30.5 (t, C-10), 24.3 (q), 22.2 (q), 20.0(s, C-4), 19.9 (q), 18.5 (t, C-3), 17.3 (q).

Isolation of *cis*-2β,3β,10,10-tetramethyl-8α- hydroxytricyclo [6.3.0.0^{2,6}] undec-5-en-4-one (anhydroarthrosporone, 21):

A fraction containing **21** (640 mg) was applied to a flash chromatography column (50 mm) and eluted with chloroform at a flow rate of 2 inches per minute. The polarity of the eluant was gradually increased by addition of acetone (ratio $(CH_3)_2CO$ / $CHCl_3$ 5:95, 1:9, 1:4). Eleven final fractions were collected. The 8th and 9th fractions were combined to give fraction F (240 mg).

Fraction F (48 mg) was further purified by flash chromatography using acetonitrile / dichloromethane (1:4). The fractions were monitored by TLC (CH_3CN / CH_2Cl_2 1:3, developmentx2) and were combined to give six fractions. The fourth fraction F-4 (18.1 mg) proved to be a single, colorless, crystalline compound which was recrystallized

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* The chemical shifts for the cyclopropane protons were assigned on the basis of their coupling constant.

from Skellysolve B / diethyl ether to give an analytically pure sample of anhydroarthrosporone **21**. m.p. 118-119°C.

TLC R_f 0.74 (acetone / benzene 2.3), 0.49 ethyl acetate / pentane 1:1, development x2).*

OR $[\alpha]_D^{23}$ +62 (*c* 2.0, $CHCl_3$).

UV (MeOH) λ_{max} 230 nm (ϵ 13700).

FTIR ($CHCl_3$, cast) 3461 (broad, strong), 1693 (strong), 1632 (strong), 1466, 1448, 1372 and 1364 (a doublet) and 872 cm^{-1}.

HREIMS m / z (formula, intensity) 234.1617 ($C_{13}H_{22}O_2$, 62.9, M^+) 219 ($C_{14}H_{19}O_2$, 32.3, $M^+ - CH_3$), 216 ($C_{13}H_{20}O$, 33.7, $M^+ - H_2O$), 201 $C_{14}H_{17}O$, 22.5), 178 ($C_{11}H_{14}O_2$, 17.3), 173 ($C_{13}H_{17}$, 23.1), 123 ($C_8H_{11}O$, 77.5, $M^+ - C_7H_{11}O$), 122 ($C_8H_{10}O$, 100.0, $C_8H_{11}O$-H), 111 ($C_7H_{11}O$, 10.3 $M^+ - C_6H_{11}O$), 95 (C_7H_{11}, 12.9, $C_8H_{11}O$-CO) and 55 (C_4H_7, 20.4, $M^+ - C_{11}H_{15}O_2$).

CIMS m / z (intensity, fragment) 252 (2.2, $M \cdot NH_4^+$) 235 (100.0, $M \cdot H^+$), 234 (20.0 $M \cdot NH_4^+ - H_2O$ or M^+).

^1H NMR (400 MHz, $CDCl_3$) δ 5.85 (1H, d, 1.3 Hz, H-5), 2.79 (1H, d, 15.8 Hz, H-7a), 2.72 (1H, dd, 1.3 and 15.8 Hz, H-7b), 2.38 (1H, dd, 9.0 and 11.0 Hz or often brt, 10 Hz, H-1), 2.32 (1H, q, 7.0 Hz, H-3), 1.87 (1H, dd, 2.0 and 14.0 Hz, H-9a), 1.72 (1H, ddd, 2.0, 9.0 and 13.0 Hz, H-11a), 1.68 (1H, d, 14.0 Hz, H-9b), 1.63 (1H, brs, OH), 1.47 (1H, dd, 11.0 and 13.0 Hz, H-11b), 1.2 (3H, s, H-12), 1.13 (3H, s, H-13), 1.11 (3H, d, 7.0 Hz, H-15) 0.94 (3H, s, H-14).

^1H NMR (C_5D_5N) δ 6.21 (1H, s, OH), 5.92 (1H, d, 2.0 Hz, H-5), 2.96 (1H, d, 15.5 Hz, H-7a), 2.80(1H, dd, 15 and 2 Hz, H-7b), 2.63 (1H, brt, 10 Hz, H-1), 2.41 (1H, q, 7.5 Hz, H-3), 2.08 (1H, dd, 14 and 2 Hz, H-9a), 1.68 (1H, d, 14Hz, H-9b), 1.65 (7H, ddd, 13, 9 and 2 Hz, H-11a), 1.45 (1H, dd, 13 and 10 Hz, H-11b), 1.40(3H, s, H-12) 1.14 (3H, d, 7.5 Hz, H-15), 1.10(3H, s, H-13) and 0.86 (3H, s, H-14).

^{13}C NMR ($CDCl_3$, 100MHz) δ 211.6 (s, C-4), 177.0(s, C-6), 122.9 (d, C-5), 92.7 (s, C-8), 63.4 (d, C-3), 57.7 (d, C-1), 55.9 (t, C-7), 53.4 (s, C-2), 44.0 (t, C-9), 43.3 (s, C-10), 41.8 (t, C-11), 30.2,** 28.2 (qx2, *gem*-dimethyl), 21.8 (q, C-2 Me), 9.5 (q, C-3 Me).

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*Compound **21** shows the same color reaction as compound **20**.
**All the quartet shifts are interchangeable.

Isolation of *cis,anti,cis*-2β,3β,10,10-tetramethyltricyclo [6.3.0.0^{2,6}] undecan-4α,6β,8α-triol (arthrosporol, 22)

Silica gel (7.4 g) in dichloromethane was packed in a 25 mL buret. A fraction (72 mg) containing **22** was applied to the column and the column was eluted with acetone / dichloromethane (3:7) at a flow rate of 0.4 mL / min. The fractions collected were monitored by TLC and combined to give nine fractions (F-1 to F-9). Fraction F-7 (41.6 mg), which contained the triol, was recrystallized from diethyl ether at 4°C for several weeks to yield an analytical sample of **22** (19 mg) as colorless needles: m.p. 163-164°C.

TLC: R'_f* 0.39 (acetone / benzene 3:2), 0.32 (acetone / benzene 2:3, developmentx2).

OR: $[\alpha]_D^{23}$ -29 (*c* 2.0, $CHCl_3$), -62.1 (*c* 1.0, MeOH).

FTIR ($CHCl_3$, cast): 3376 (broad, strong), 2951, 2933, 1456, 1380 and 1372 (doublet), 1088 and 1012 cm^{-1}.

HREIMS: m / z (formula, intensity) 236.1773 ($C_{15}H_{24}O_2$, 15.8, $M^{\cdot} - H_2O$), 218 ($C_{15}H_{22}O$, 100.0, $M^{\cdot} - 2H_2O$), 203 ($C_{14}H_{19}O$, 30.7, $C_{13}H_{22}O$-CH_3), 190 ($C_{14}H_{22}$, 7.8, $C_{13}H_{22}O$-CO), 182 ($C_{11}H_{18}O_2$, 38.0, $M^{\cdot} - C_4H_8O$), 174 ($C_{13}H_{18}$, 32.8) 164 ($C_{11}H_{16}O$, 18.0, $C_{11}H_{18}O_2$-H_2O), 150 ($C_{11}H_{18}$, 54.0), 139 ($C_9H_{15}O$, 32.2), 135 ($C_{10}H_{15}$, 32.6), 127 ($C_7H_{11}O_2$, 32.4, $M^{\cdot} - C_8H_{13}O$), 126 ($C_8H_{14}O$, 2.3 and $C_7H_{10}O_2$, 8.7), 125 ($C_7H_9O_2$, 16.1), 123 ($C_8H_{11}O$, 16.5, $M^{\cdot} - C_7H_{13}O_2$), 121 (C_9H_{13}, 24.7), 110 ($C_7H_{10}O$, 25.3), 109 (C_8H_{13}, 38.7 and C_7H_9O, 22.4), 107 (C_8H_{11}, 34.3), 95 (C_7H_{11}, 31.0), 83 (C_5H_7O, 54.3, $C_7H_{10}O$-CH_3), 69 (C_5H_9, 38.4) and 55 (C_4H_7, 65.2 and C_3H_3O, 15.9).

CIMS: m / z (intensity, fragment) 272 (100.0, $M{\cdot}NH_4^+$) 254 (56.9, $M{\cdot}NH_4^+$-18), 237 (14.6 $M{\cdot}H^+$-H_2O).

^1H NMR (400 MHz, $CDCl_3$): δ 3.94 (1H, td, 9.0 and 5.0 Hz, H-4), 2.40 (1H, dd, 8.0 and 12.0 Hz, H-1), 2.32 (1H, d, 15.0 Hz, H-7a), 2.22 (1H, d, 15.0 Hz, H-7b), 2.06 - 1.96 (3H, m) 1.86 (1H, d, 13 Hz, H-9a), 1.77 (1H, brs, OH), 1.70 (1H, dd, 2.0 and 13.0 Hz, H-9b),

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*R'_f was calculated using anhydroarthrosporone as the reference compound.

1.64 (1H, brs, OH), 1.54 (1H, brt, 12 Hz, H-11a), 1.47 (1H, ddd, 2.0, 8.0 and 12.0 Hz, H-11b), 1.11 (3H, s, H-12), 1.04 (3H, s, H-13), 1.02 (3H, d, 7.0 Hz, H-15), 0.76 (3H, s, H-14).

^{13}C NMR (100 MHz, CD_3OD): δ 91.0 (s, C-6 or C-8), 90.3 (s, C-8 or C-6), 76.8(d, C-4), 60.9 (d, C-3 or C-1), 58.5 (t, C-7 or C-5), 58.2 (t, C-5 orC-7), 56.3 (s, C-2), 52.1 (d, C-1 orC-3), 49.0 (t, C-11 orC-9), 45.3 (t, C-9 or C-11), 40.0 (s, C-10), 30.7, 28.6, 12.9 and 12.5 / each q, 4xCH_3).

Isolation of *cis*-2β,3α, 10,10-tetramethyl- 9α hydroxytricyclo [6.3.0.0] undecac-1(8)-en-4,11-dione (anhydroarthrosporodione, 23) and the minor *cis,anti,cis* -2β, 3β, 10,10-tetramethyl- 4α- 4-O-acetyl-tricyclo [6.3.0.0^{2,6}] undecan-6β,8α-diol (O-acetylarthrosporol, 24)

A fraction (82.5 mg) containing compounds **23** and **24** was subjected to flash chromatography over silica gel (10 mm) by elution with acetone / dichloromethane (2:7). Fraction D (34.5 mg) contained compound **23** and a small amount of compound **24**. Fraction D (18 mg) was repurified by preparative TLC (silica gel, acetone / dichloromethane 2:7, developmentx2) to give compound **23** as a colorless oil* (8.5 mg) and compound **24**.

Diketone 23

TLC: R'_f 0.74 (acetone / benzene 2:7); 0.47 (ethyl acetate / pentane 1:2, developmentx2): dark purple spot (reagent A).

OR: $[\alpha]_D^{23}$ -80.1 (*c* 2.5, $CHCl_3$).

UV (MeOH): λ_{max} 242 nm (ε 7712); uv (MeOH + 0.1 *N* HCl). λ_{max} 242 nm (ε 8142); UV (MeOH + 0.1 *N* NaOH): λ_{max} 242 nm (ε 10594).

FTIR ($CHCl_3$, cast): 3450 (broad, strong), 1738 (strong) 1700 (strong), 1688 (strong), 1637 (strong), 1374 and 1089 cm^{-1}.

HREIMS: m / z (formula, intensity) 248 ($C_{15}H_{20}O_3$, 42.0, M^+), 233 ($C_{14}H_{17}O_3$, 100.0, $M^+ - CH_3$), 230 ($C_{15}H_{18}O_2$, 4.2, $M^+ - H_2O$), 215 ($C_{14}H_{15}O_2$, 5.4, $C_{14}H_{17}O_3$-H_2O), 192 ($C_{12}H_{16}O_2$,

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*The colorless oil turns yellow at room temperature after some period of time.

11.4), 177 ($C_{11}H_{13}O_2$, 18.6), 173 ($C_{12}H_{13}O$, 7.3), 131 ($C_{10}H_{11}$, 12.6), 91 (C_7H_7, 16.7).

CIMS: m/z (intensity, fragment) 266 (100.0, $M{\cdot}NH_4^+$) 248 (8.1 $M{\cdot}NH_4^+$-18), 235 (13.7, $M{\cdot}H^+$-H_2O),

^{1}H NMR (400 MHz, $CDCl_3$): δ 4.51 (1H, 6.7 and 2.2 Hz, H-9, 2.93 (1H, m, H-6), 2.83 (1H, ddd, 2.2, 9.0 and 16.0 Hz, H-7a), 2.60 (1H, dd, 9.0 and 19.0 Hz, H-5a), 2.48 (1H, qd, 7.0 and 1.5 Hz, H-3), 2.39 (1H, ddd, 1.5, 7.0 and 19.0 Hz, H-5b), 2.21 1H, ddd, 1.8, 7.0 and 16.0, H-7b), 1.87 (1H, d, 7.0 Hz, exchangeable with D_2O), 1.19 and 1.18 (2xs, two methyls), 1.17 (3H, d, 7.0Hz, H-15), 1.09 (3H, s, methyl).

^{13}C NMR (100 MHz, $CDCl_3$): δ 218.8 (s, C-4), 205.8 (s, C-11) 185.0 (s, C-8), 145.1 (s, C-1), 75.1 (d, C-9), 54.3 (s, C-10) 51.0 (d, C-3, or C-6), 49.9 (d, C-6 or C-3), 42.1 (s, C-2), 41.7 (t, C-5 or C-7), 31.7 (t, C-7 or C-5), 23.6, 20.2, 18.5 and 9.3 (4x9, methyls).

Arthrosporol monoacetate 24:

M.p. 132-135°C (diethyl ether, Skellysolve B).

R'_f 0.70 (acetone/benzene 2:7), 0.44 (ethyl acetate/*n*-pentane 1:2, developmentx2).

OR: $[\alpha]_D^{23}$ -60.9 (*c* 1.4 $CHCl_3$).

FTIR ($CHCl_3$, cast): 3540 (sharp), 3440(broad), 1720 (strong), 1261 (strong) and 1021 (sharp) cm^{-1}.

HREIMS: m/z (formula, intensity) 278.1885 ($C_{17}H_{26}O_3$, 0.2, $M^+ - H_2O$), 263 ($C_{16}H_{23}O_3$, 0.2, $C_{17}H_{26}O_3$-CH_3), 236 ($C_{15}H_{24}O_2$, 3.5, M^+ – HOAc), 218 ($C_{15}H_{22}O$, 100.0, M^+ – HOAc-H_2O), 203 ($C_{14}H_{19}O$, 18.0), 200 ($C_{15}H_{20}$, 0.8, M^+ – HOAc-$2H_2O$), 160 ($C_{12}H_{16}$, 13.5), 139 ($C_9H_{15}O$, 12.7), 136 ($C_{10}H_{16}$, 40.5) 109 ($C_7H_{10}O$, 24.9), 109 (C_8H_{13}, 22.0 and C_7H_9O, 26.2) and 55 (C_4H_7, 23.4).

CIMS: m/z (intensity, fragment) 314 (100.0, $M{\cdot}NH_4^+$) 296 (4.6, $M{\cdot}NH_4^+$ - 18) and 236 (2.0, M^+ - 60).

^{1}H NMR (400 MHz, $CDCl_3$): δ 4.84 (1H, td, 9.0 and 4.0 Hz, H-4), 2.41 (1H, dd, 8.0 and 11.0 Hz, H-1), 2.27 (1H, d, 14.0 Hz, H-7a), 2.23 (1H, d, 14.0 Hz, H-7b), 2.21 (1H, dq, 9.0 and 7.0 Hz, H-3), 2.13 (1H, dd, 14.0 and 9 Hz, H-5a), 2.03 (3H, s, CH_3CO-), 1.96 (1H, dd, 14.0 and 4.0 Hz, H-5b), 1.91 (1H, d, 13.8 Hz, H-9a), 1.73 (1H, dd, 13 and 2.5 Hz, H-9b),

1.60 (1H, t, 12 Hz, H-11a), 1.51 (1H, ddd, 13.0, 8.0 and 2.5 Hz, H-11b), 1.09 (3H, s, methyl), 1.04 (3H, s, methyl), 0.96 (3H, d, 7.0 Hz, H-12), 0.78 (3H, s, methyl).

***cis,anti,cis*-2β,3β,10,10-Tetramethyl-8α-acetoxy- 6β-hydroxytricyclo [6.3.0.0^{2,6}] undecan-4-one (O-acetylarthrosporone, 27)**

Arthrosporone **20** (2.4 mg, 0.0096 mmole) was dissolved in triethylamine (2 mL). Dimethylaminonypyridine (DMAP, 1.5 mg) and acetic anhydride (Ac_2O, 4 drops) were added and the mixture was allowed to stir at room temperature for 3 days. The solvents were removed *in vacuo* and the residue was partitioned between ethyl acetate (20 mL) and 5% aqueous HCl (5 mL), washed with water (5 mL), dried over sodium sulfate (Na_2SO_4), and concentrated to dryness. The residue was purified by chromatography over silica gel (acetone / benzene 1:9) to give analytically pure monoacetate **27**, m.p. 120-122°C.

TLC: R'_f 0.58 (ethyl acetate / pentane 1:2, developmentx2).

OR: $[\alpha]_D^{23}$ -37.6° (*c* 5.0, $CHCl_3$).

IR ($CHCl_3$, cast): 3449 (broad), 1736 (strong), 1382 and 1368 (doublet), 1249 (strong), 1017 cm^{-1}.

HREIMS: m / z (formula, intensity) 294 ($C_{17}H_{26}O_4$, 1.1, M^+), 252 ($C_{15}H_{24}O_3$, 1.3, $M^+ - C_2H_2O$), 234 ($C_{13}H_{22}O_2$, 32.1, $M^+ - HOAc$), 216 ($C_{15}H_{20}O$, 3.0, $M^+ - HOAc-H_2O$), 192 ($C_{13}H_{20}O$, 100.0, $M^+ - HOAc-C_2H_2O$), 125 ($C_7H_9O_2$, 92.3), 110 (C_8H_{14}, 30.5); m / z calcd. for $C_{17}H_{26}O_4$ 294.1824; found 294.1818.

^1H NMR ($CDCl_3$): δ 2.83 (1H, dd, 12 and 8.5 Hz, H-1), 2.81 (1H, d, 16 Hz, H-7a), 2.64 (1H, dd, 19 and 1 Hz, H-5a), 2.34 (1H, qd, 7 and 1 Hz, H-3), 2.28 (1H, dd, 15 and 3 Hz, H-9a), 2.24 (1H, d, 15.5 Hz, H-7b), 2.22 (1H, d, 19 Hz, H-5b), 1.98 (3H, s, CH_3CO), 1.86 (1H, d, 15 Hz, H-9b), 1.69 (1H, t, 12 Hz, H-11a), 1.56 (1H, ddd, 12, 8.5 and 3 Hz, H-11b), 1.47 (1H, brs, D_2O-exchangeable), 1.08 (3H, s, H-14), 1.06 (3H, d, 7 Hz, H-15), 1.04 (3H, s, H-12), 0.89 (3H, s, H-13).

^1H NMR (C_5D_5N): δ 3.10 (1H, brd, 15.5 Hz, H-7a), 2.97 (1H, br dd, 12 and 8 Hz, H-1), 2.69 (1H, brd, 19 Hz, H-5a), 2.52 (1H, brq, H-3), 2.46 (1H, brd, 15 Hz, H-9a), 2.45 (1H, brd, 16 Hz, H-7b), 2.44 (1H, brd, 19 Hz, H-5b), 2.15 (1H, brd, 15 Hz, H-9b), 2.00 (1H, brt, 12 Hz,

H-11a), 1.95 (3H, s, $C\underline{H}_3CO$), 1.58 (1H, ddd, 12, 8 and 3 Hz, H-11b), 1.11 (3H, d, 7 Hz, H-15), 1.09 (3H, s, H-14) 1.07 (3H, s, H-13), 1.01 (3H, s, H-12).

cis-2β,3β,10,10-Tetramethyl-8α-acetoxytricyclo [6.3.0.0^{2,6}] undec-5-en-4-one (O-acetylanhydroarthrosporone, 34)

A mixture of anhydroarthrosporone **21** (5.7 mg, 0.0245 mmole), dimethylaminopyridine and acetic anhydride (5 drops) in triethylamine (2 mL) was allowed to stir at room temperature for 7 h. The solvents were evaporated *in vacuo* and the residue was partitioned between dichloromethane (20 mL) and 5% aqueous hydrochloric acid solution (2x2 mL). The organic phase was dried (Na_2SO_4) and evaporated to give crude acetate (5.1 mg). Silica gel column chromatography (acetone / chloroform 3:97) of the crude acetate gave pure acetate **34** as a colorless liquid.*

TLC: R'_f 1.21 (acetone / benzene 1:8), 1.00 (ethyl acetate / pentane 1:2).

OR: $[\alpha]_D^{23}$ +78.0 (*c* 6.4, $CHCl_3$).

FTIR ($CHCl_3$, cast), 1728, 1706 (both strong), 1638, 1241 (strong) cm^{-1}.

UV (MeOH): λ_{max} 228 nm (ϵ 8273).

HREIMS: m / z (formula, intensity) 276 ($C_{17}H_{24}O_3$, 1.8, M^+), 234 ($C_{15}H_{22}O_2$, 4.0, $M^+ - CH_2CO$), 216 ($C_{15}H_{20}O$, 100.0 $M^+ - AcOH$), 201 ($C_{14}H_{17}O$, 38.5), 173 ($C_{13}H_{13}$, 36.2, $C_{14}H_{17}O\text{-}CO$), 122 ($C_8H_{10}O$, 26.5); m / z calcd. for $C_{17}H_{24}O_3$: 276.1719; found 276.1724.

^1H NMR ($CDCl_3$): δ 5.75 (1H, d, 2.2 Hz, H-5), 3.30 (1H, d, 17 Hz, H-7a), 2.74 (1H, brdd, ** 17, and 2.2 Hz, H-7b), 2.68 (1H, brt, 10 Hz, H-1), 2.43 (1H, brd,* 15 Hz, H-9a), 2.33 (1H, q, 7.5 Hz, H-3), 1.97 (3H, CH_3CO), 1.69 (1H, ddd, 12.5, 9 and 2.2 Hz, H-11a), 1.64 (1H, d, 15 Hz, H-9b), 1.45 (1H, dd, 12.5 and 9.5 Hz, H-11b), 1.11 (3H, s, H-13), 1.10 (3H, d, 7.5 Hz, H-15), 1.09 (3H, s, H-14), 0.93 (3H, s, H-12).

(-)-[5-(4',4'-dimethyl-2'-oxycyclopentyl)-1,4,5-trimethylcyclopent- 3-en-2-one]

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* The liquid solidifies very slowly after a long period of time: m.p. 76-7°C.
** Resolution enhancements showed that the proton at δ 2.74 was a ddd (16.0, 2.4 and 1.2 Hz) while the proton at δ 2.43 displayed a ddd (14.5, 2.2 and 1.5 Hz) pattern.

(bicyclodiketone, 39)

A solution of **21** (4.1 mg, 0.0176 mmole) in dry benzene (2 mL) was flushed for 10 min with nitrogen. Sodium hydride (excess) was added as a suspension in benzene to the solution. The mixture was allowed to stir at room temperature under a nitrogen atmosphere for 5 h. The mixture was diluted with benzene (5 mL), quenched with 10% aqueous acetic acid, then allowed to stir until the excess of sodium hydride dissolved. The benzene layer was washed with brine, dried ($MgSO_4$) and evaporated *in vacuo*. The crude (3.9 mg) was purified by chromatography over silica gel (chloroform) to give pure bicyclic compound **39** as a crystalline material.

M.p. 55-56°C (ether, slow evaporation).

TLC: R'_f 1.29 (acetone/benzene 1:8), 1.00 (ethyl acetate/pentane 1:3, developmentx2); reagent A does not char the compound **39**.

OR: $[\alpha]_D^{23}$ -202° (*c* 1.95, $CHCl_3$).

FTIR ($CHCl_3$, cast): 1738, 1702 and 1625 cm^{-1}.

UV (MeOH): λ_{max} 223 mm (ϵ 13400).

HREIMS: m/z calcd. for $C_{15}H_{22}O_2$: 234.1614; found: 234.1623 (59.4, M^+), 219 ($C_{14}H_{19}O_2$, 6.8) 150 ($C_{10}H_{14}O$, 36.5), 124 ($C_8H_{12}O$, 35.9, $M^+ \cdot C_7H_{10}O$), 12.3 ($C_8H_{11}O$, 100.0), 122 ($C_8H_{10}O$, 89.6), 112 ($C_7H_{12}O$, 26.2, $M^+ - C_8H_{12}O$), 97 (C_6H_9O, 15.1, 112-CH_3), 95 (C_7H_{11}), 50.2).

^1H NMR ($CDCl_3$): δ 5.92 (1H, q, 1.6 Hz, H-5)* 2.58 (1H, ddd, 12.5, 8.5 and 1.5 Hz, H-1), 2.20 (1H, ddd, 18.0, 2.4 and 1.5 Hz, H-9a), 2.08 (1H, q, 7.5 Hz, H-3), 2.05 (3H, d, 1.6 Hz, H-7), 2.04 (1H, dd, 18.0 and 1 Hz, H-9b), 1.67 (1H, ddd, 13.0, 8.5 and 2.5 Hz, H0-11a), 1.45 (3H, s, H-14), 1.35 (1H, brt, 12.5 Hz, H-11b), 1.19 (3H, s, H-13), 1.08 (3H, d, 7.5 Hz, H-15) and 1.04 (3H, s, H-12).

Selective reduction of acetoxyanhydroarthrosporone

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* The coupling constant for long range couplings were obtained by resolution enhancement experiments.

The unsaturated acetate **34** (5 mg, 0.018 mmole) was dissolved in methanol (1.5 mL). Cerium(III) chloride (2.2 mg) and sodium borohydride (10 mg in methanol) were added and the mixture was allowed to stir at room temperature. After 1 h, the mixture was worked up as usual and the crude product was purified by chromatography over silica gel (acetone / dichloromethane 5:95) to give three alcohols (**46, 47, 48**).

***cis*-2β,3β, 10,10-Tetramethyl-8α acetoxytricyclo [6.3.0.0^{2,6}] undec-5-en-4α-ol (unsaturated acetoxy alcohol, 46)**

TLC: R'_f 0.83, acetone / benzene 1:3), 0.50(ethyl acetate / pentane 1:2), greyish blue spot (reagent A).

FTIR ($CHCl_3$, cast): 3400 (br), 1732 (strong), 1676 (weak), 1242 (strong), 1017 cm^{-1}.

HREIMS: m / z calcd. for $C_{17}H_{26}O_3$ (M^+): 278.1875; found 278.1873 (0.8, M^+), 218 ($C_{15}H_{22}O$, 100.0, M^+ – HOAc) 203 ($C_{14}H_{19}O$, 34.8, 218-CH_3), 200 ($C_{15}H_{20}$, 4.4, 203-H_2O), 106 (C_8H_{10}, 84.5), 91 (C_7H_7, 20.3).

CIMS: m / z 296 (6.5, $M{\cdot}NH_4^+$), 278 (5.6, $M{\cdot}NH_4^+$-18), 201 (100.0, $M{\cdot}H^+$-HOAc-H_2O).

^{1}H NMR ($CDCl_3$): δ 5.29 (1H, brs, H-5), 4.56 (1H, brm, H-4), 3.02 (1H, dd, 17.0 and 1 Hz, H-7a), 2.57 (1H, brdd, 10 and 9 Hz, H-1), 2.33 (1H, dd, 17 and 2.2 Hz, H-7b), 2.29 (1H, dq, 15 and 2.4 Hz, H-9a), 2.00 (3H, s, CH_3CO), 1.81 (1H, dq, 6 and 7.4 Hz, H-3), 1.53 (1H, d, 15 Hz, H-9b), 1.52 (1H, ddd, 13, 9 and 24 Hz, H-11a), 1.32 (1H, dd, 13 and 10 Hz, H-11b), 1.09 (3H, d, 7.5 Hz, H-15), 1.05 (3H, s, H-14), 1.03 (3H, s, H-12), 0:80 (3H, s, H-13).

^{1}H NMR (C_5D_5N): δ 6.43 (1H, brd, 4.2 Hz, CHOH), 5.66 (1H, brd, 2 Hz, H-5), 4.94 (1H, m, H-4), 3.29 (1H, brd, 16 Hz, H-7a), 2.70 (1H, brt, 10 Hz, H-1), 2.52 (1H, brdd, 15 and 2 Hz, H-9a), 2.46 (1H, brdd, 15.6 and 2.2 Hz, H-7b), 2.26 (1H, dq, 6 and 7.4 Hz, H-3), 1.93 (3H, s, CH_3CO), 1.62 (1H, d, 15 Hz, H-9b), 1.46 (1H, ddd, 12.8, 9 and 2 Hz, H-11a), 1.33 (1H, dd, 12.8 and 11 Hz, H-11b), 1.26 (3H, d, 7.5 Hz, H-15), 1.06 (3H, s, H-12), 1.02 (3H, s, H-14), 0.83 (3H, s, H-13).

***cis,anti,cis*-2β,3β, 10,10-Tetramethyl-8α- acetoxytricyclo [6.3.0.0^{2,6}] undecan-4β-ol (least polar and minor acetoxyalcohol, 47)**

R'_f 0.93 (acetone/benzene 1:3), 0.68 (ethyl acetate/ pentane 1:2), reddish spot turning dark blue overnight (reagent A).

FTIR ($CHCl_3$, cast): 3464 (broad), 1732 (shoulder) 1724 (strong), 1367, 1245 (strong) and 1016 cm^{-1}.

HREIMS: m/z 220 ($C_{15}H_{24}O$, 31.5, M^+ – HOAc), 202 ($C_{15}H_{22}$, 5.0, 220-H_2O), 16 ($C_{13}H_{20}$, 22.4), 161 ($C_{12}H_{17}$, 55.9), 148 ($C_{11}H_{16}$, 64.8), 123 ($C_8H_{11}O$, 12.8), 110 ($C_7H_{10}O$, 100.0), 107 (C_8H_{11},; 25.9).

CIMS: m/z 298 (60.2, M·H NH_4^+): thus 280 (0.7, M^+)

^{1}H NMR ($CDCl_3$): δ 4.31 (1H, brt, 6.0 and 3.0 Hz, H-4), 2.67 (1H, brdd, 12.5 and 8.0 Hz, H-1), 2.37 (1H, dd, 10 and 9.0 Hz, H-7a), 2.37-2.30 (2H, m, H-3 and H-6), 2.23 (1H, brs, CHO<u>H</u>), 1.96 (1H, ddd, 14, 6, and 3.6 Hz, H-5a), 1.88 (1H, m, H-5b), 1.80 (1H, d, 15 Hz, H-9a), 1.53 (1H, ddd, 12.5, 8 and 3 Hz, H-11a), 1.30 (1H, t, 12.5 Hz, H-11b), 1.4 (3H, s, H-13), 1.02 (3H, s, H-12), 1.00 (3H, d, 7.2 Hz, H-15), 0.95 (3H, s, H-14).

^{1}H NMR (C_5D_5N): δ 5.82 (1H, d, 2 Hz, CHO<u>H</u>), 4.52 (1H, m, H-4), 2.84 (1H, dd, 12.5 and 8 Hz, H-1) 2.46 (1H, dd, 15 and 9 Hz, H-7a), 2.34 (1H, dq 4 and 8 Hz, H-3), 2.27 (1H, dd, 15 and 2.5 Hz, H-9a), 2.13 (1H, ddd, 13.5, 8 and 3 Hz, H-5a), 2.11 (1H, dd, 14.5 and 7 Hz, H-7a), 2.01 (3H, s, C<u>H</u>$_3$CO), 2.01 (1H, ddd, 14.2, 6 and 4 Hz, H-5b), 1.85 (1H, d, 14.8 Hz, H-9b), 1.49 (1H, ddd, 12.5, 8 and 2.5 Hz, H-11a), 1.31 (1H, t, 12.5 Hz, H-11b), 1.25 (3H, d, 8 Hz, H-15), 1.21 (3H, s, H-14), 1.08 (3H, s, H-12), 1.02 (3H, s, H-13).

***cis,anti,cis*-2β,3β-Tetramethyl-8α-acetoxytricyclo [6.3.0.0^{2,6}] undecan-4α-ol (most polar and major acetoxyalcohol, 48)**

TLC: R'_f 0.65 (acetone/benzene 1:3), 0.36 (ethyl acetate/pentane 1:2), reddish spot turning dark blue overnight (reagent A/char technique).

FTIR ($CHCl_3$, cast): 3400 (broad), 1732 (strong), 1713 (shoulder), 1383 and 1366

(doublet), 1243 (strong), 1087 (strong) and 1016 (strong) cm^{-1}.

HREIMS: m/z 238 ($C_{15}H_{26}O_2$, 0.5, $M^+ - C_2H_2O$), 220 ($C_{15}H_{24}O$, 40.9, $M^+ - HOAc$), 202 ($C_{15}H_{22}$, 66.0, 220-H_2O), 187 ($C_{14}H_{19}$, 23.4), 176 ($C_{13}H_{20}$, 16.3), 161 ($C_{12}H_{17}$, 52.0), 148 ($C_{11}H_{16}$, 100.0), 111 ($C_7H_{11}O$, 18.7), 108 (C_8H_{12}, 34.0), 93 (C_7H_9, 25.3).

^1H NMR ($CDCl_3$): δ 3.89 (1H, td, 9 and 6 Hz, H-4), 2.67 (1H, bdd, 12.5 and 8 Hz, H-1), 2.43 (1H, dd, 17.5 and 11 Hz, H-7a), 2.29 (1H, m, H-6), 2.11 (1H, dd, 15 and 3 Hz, H-9a), 2.09 (1H, m, H-5a), 2.08 (1H, dd, 18 and 8 Hz, H-7b), 2.02 (3H, s, CH_3CO), 1.97 (1H, dq, 9 and 7.3 Hz, H-3), 1.75 (1H, bd, 15 Hz, H-9b), 1.48 (1H, ddd, 12.6, 8 and 3 Hz, H-11a), 1.38 (1H, ddd, 13, 6 and 3.5 Hz, H-5b), 1.22 (1H, t, 12.5 Hz, H-11b), 1.05 (6H, s, H-12 and H-13), 1.03 (3H,d, 7.2 Hz, H-15), 0.80 (3H, s, H-14).

^1H NMR (C_5D_5N): δ 6.26 (1H, bd, 4.2 Hz, CHOH), 4.18 (1H, m, H-4), 2.85 (1H, dd, 12 and 8 Hz, H-1), 2.59 (1H, dd, 15 and 9 Hz, H-7a), 2.46 (1H, dd, 14 and 8 Hz, H-7b), 2.40 (1H, m, H-6), 2.38 (1H, dq, 8 and 7 Hz, H-3), 2.29 (1H, dd, 15 and 2.5 Hz, H-9a), 2.04 (1H, ddd, 15.8 and 4 Hz, H-5a), 1.88 (3H, s, CH_3CO), 1.83 (1H, d, 15 Hz, H-9b), 1.75 (1H, ddd, 14.5, 6 ad 4 Hz, H-5b), 1.47 (1H, ddd, 12.8 and 2.5 Hz, H-11a), 1.25 (1H, t, 12 Hz, H-11b), 1.22 (3H, d, 7 Hz, H-15), 1.09 (3H, s, H-12), 1.03 (3H, s, H-13), 0.83 (3H, s, H-14).

cis,anti,cis-2β,3β, 10,10- Tetramethyl-8α- hydroxytricyclo [6.3.0.0^{2,6}] undecan-4-one (51)

A mixture of **21** (10 mg, 0.043 mmole), acetic acid (10% in methanol, 4 drops) and activated 10% Pd/C in methanol (2 mL) was stirred at room temperature under an atmosphere of hydrogen contained in a small balloon. After 30 minutes the mixture was filtered through a celite and the celite washed with methanol. The combined filtrate was concentrated to dryness under reduced pressure to give saturated ketone **51** in quantitative yield. An analytically pure sample was prepared by recrystallization(Skellysolve B/diethyl ether); m.p. 115-116°C.

TLC: R'_f 0.82 (acetone/benzene 2:7), 0.70 (ethyl acetate/pentane 1:2, developmentx2), yellow spot.

FTIR (CH_2Cl_2, cast): 3502 (strong), 1727 (strong) cm^{-1}.

HREIMS: m/z calcd. for $C_{15}H_{24}O_2$: 236.1772, found: 236.1773 (M^+); m/z (formula, intensity) 236 ($C_{15}H_{24}O_2$, 16.4, M^+), 221 ($C_{14}H_{21}O_2$, 8.6, $M^+ - CH_3$), 218 ($C_{15}H_{22}O_0$, 19.8, $M^+ - H_2O$), 203 ($C_{14}H_{19}O$, 9.01, 218-CH_3), 176 ($C_{13}H_{20}$ 70.7, $M^+ - CH_2CO-H_2O$), 161 ($C_{12}H_{17}$, 33.3), 124 ($C_8H_{12}O$, 38.9, $M^+ - C_7H_{12}O$), 110 ($C_7H_{10}O$, 100.0, $M^+ - C_8H_{14}O$), 109 (C_8H_{13}, 65.4), 83 (C_5H_7O, 5.5).

^1H NMR ($CDCl_3$): δ 2.54 (2H, m, H-1 and H-3),* 2.39 (1H, brdd, 18 and 8 Hz, H-5a), 2.31 (1H, m, H-6), 2.28 (1H, dd, 15 and 8 Hz, H-7a), 2.19 (1H, brd, 18 Hz, H-5b), 1.78 (1H, dd, 14 and 2.8 Hz, H-9a), 1.68 (1H, brd, 14 Hz, H-9b), 1.6 (1H, dd, 15 and 10 Hz, H-7b), 1.63 (1H, brs, OH), 1.60 (1H, ddd, 12.5, 7 and 2.8 Hz, H-11a), 1.29 (1H, t, 12.5 Hz, H-11b), 1.14 (3H, s, H-14), 1.07 (3H, s, H-13), 0.98 (3H, d, 7 Hz, H-15), 0.77 (3H, s, H-12).

Reduction of ketol 51

The ketol **51** (8 mg, 0.034 mmole) in methanol (3 mL) was allowed to stir at room temperature in the presence of an excess of sodium borohydride for 1 h. Acetic acid (5 drops) was added and the mixture was allowed to stir for 5 min. Solvents were removed and the residue was partitioned between water (5 mL) and ethyl acetate (3x10 mL). The organic phase was dried and concentrated to give a diasteroisomeric mixture of alcohols (**52, 55**). The two alcohols were separated by a mini silica gel column chromatography (acetone / dichloromethane 1:9).

***cis,anti,cis*-2β,3β, 10,10- Tetramethyltricyclo [6.3.0.0^{2,6}] undecan-4α,8α-diol (most polar and major diol, 52)**

m.p. 139-140°C (CH_2Cl_2 / Skelly B).

TLC: R'_f 0.14 (acetone / benzene 2:3); 0.22 (ethyl acetate / pentane 1:1). It displays the same colour reaction (reagent A) as the minor diol.

FTIR ($CHCl_3$, cast): 3347 (broad, strong), 2953, 2932 (both strong), 2869, 1040 (strong)

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* A broad quarter (J = 7.0 Hz) overlaps a broad doublet of doublets (J = 12.5 and 7.0 Hz).

cm^{-1}.

HREIMS: m/z (formula, intensity) 220 ($C_{15}H_{24}O$), 13.2 $M^{\cdot} - H_2O$), 205 ($C_{14}H_{21}O$, 6.3, 220-CH_3), 202 ($C_{15}H_{22}$, 50.0, $M^{\cdot} - 2H_2O$), 179 ($C_{12}H_{19}O$, 32.4), 166 ($C_{11}H_{18}O$, 56.3), 148 ($C_{11}H_{16}$, 100.0), 120 (C_9H_{12}, 26.7), 111 ($C_7H_{11}O$, 54.5), 109 (C_8H_{13}, 68.8; C_7H_9O, 42.9), 108 (C_9H_{12}, 95.5), 95 (C_7H_{11}, 52.1), 55 (C_4H_7, 41.8); m/z calcd. for $C_{15}H_{24}O_2$: 238; found 238 by CI (m/z 238 ($M^{\cdot}$, 63.9).

^1H NMR ($CDCl_3$): δ 3.84 (1H, td, 9 and 6.5 Hz, H-4) 2.35 (1H, brdd, 12 and 8 Hz, H-1), 2.29 (1H, dt, 12 and 8 Hz, H-5a), 2.15 (1H, dd, 14 and 9 Hz, H-7a), 2.05 (1H, m, H-6), 2.04 (1H, dq, 9 and 7 Hz, H-3), 1.78 (1H, brdd, 14 and 6.5 Hz, H-7b), 1.70 (1H, d, 14 Hz, H-9a), 1.65 (1H, dd, 14 and 2 Hz, H-9b), 1.50 (1H, dt, 6.5 and 12 Hz, H-5b), 1.49 (1H, ddd, 12, 8 and 2 Hz, H-11a), 1.25 (1H, t, 12 Hz, H-11b), 1.12 (3H, s, H-12), 1.04 (3H, s, H-13), 1.00 (3H, d, 7 Hz, H-15), 0.78 (3H, s, H-14).

Deacetylation of acetoxyalcohol 48

Acetate **48** (5 mg) was dissolved in 10% ethanolic potassium hydroxide (3 mL) and the mixture was allowed to stir at 80°C (oil-bath). After 20 min, the mixture was cooled in an ice-water bath and quenched with 20% HCl. Most of the ethanol was removed *in vacuo*. The residue was diluted with water (2 mL) and exhaustively extracted with ethyl acetate. The ethyl acetate extract was dried and concentrated to give a yellow gum in quantitative yield. Purification by chromatography over silica gel (acetone/dichloromethane 1:4) gave diol **53** (cotton-like material) m.p. 138-140°C.

TLC: R'_f 0.16 (ethyl acetate/pentane 1:1), 0.22 (acetone/benzene 1:4). It charred red then turned dark blue overnight (reagent A).

FTIR ($CHCl_3$, cast) 3345 (broad, strong), 2952 2933 (both strong), 2869, 1045 (strong) cm^{-1}.

HREIMS: m/z (formula, intensity) 220 ($C_{15}H_{24}O$, 21.9, $M^{\cdot} - H_2O$), 205 ($C_{14}H_{21}O$, 8.6), 202 ($C_{15}H_{22}$, 69.4, $M^{\cdot} - 2H_2O$), 187 ($C_{14}H_{19}$, 25.3), 179 ($C_{12}H_{19}O$, 40.7), 166 ($C_{11}H_{18}O$, 62.1), 163 ($C_{11}H_{15}O$, 18.6), 148 ($C_{11}H_{16}$, 100.0), 123 ($C_8H_{11}O$, 15.1), 120 (C_9H_{12}, 18.8), 111 ($C_7H_{11}O$, 33.4), 109 (C_8H_{13}, 42.3; C_7H_9O, 26.5), 108 (C_9H_{12}, 62.2), 95 (C_7H_{11}, 28.6), 55

(C_4H_9, 41.8).

^1H NMR ($CDCl_3$): δ 3.84 (1H, td, 9 and 6.5 Hz), 2.35 (1H, dd, 12 and 8 Hz), 2.29 (1H, dt, 12 and 8 Hz), 2.15 (1H, dd, 13.5 and 9 Hz), 2.05 (2H, m), 1.78 (1H, brdd, 13.5 and 6.5 Hz), 1.70 (1H, d, 14 Hz), 1.65 (1H, dd, 14 and 2 Hz), 1.50 (1H, dt, 6.5 and 12 Hz), 1.49 (1H, brddd, 12, 8 and 2 Hz), 1.25 (1H, brt, 12 Hz), 1.12 (3H, s), 1.04 (3H, s), 1.00 (3H, d, 7 Hz), 0.76 (3H, s).

***cis,anti,cis*-2β,3β, 10,10- Tetramethyltricyclo [6.3.0.0^{2,6}] undecan-4β,8α-diol (least polar and minor diol, 55) as a waxy material**

TLC: R'_f 0.44 (ethyl acetate / pentane 1:1), 0.48 (acetone / benzene 1:4): a reddish spot turning into a dark blue spot (reagent A / char technique).

^1H NMR ($CDCl_3$): δ 4.29 (1H, td, 6 and 2.5 Hz, H-4), 2.31 (1H, brdd, 12 and 8 Hz, H-1), 2.27 (1H, m, H-6) 2.15 (1H, quintet, 7 Hz, H-3), 2.06 (1H, dd, 15 and 9 Hz, H-7a), 2.03 (1H, ddd, dd, 14, 6 and 5 Hz, H-5a), 1.84 (1H, ddd, 14, 8 and 2.5 Hz, H-5b), 1.70 (1H, d, 14 Hz, H-9a), 1.61 (1H, dd, 14 and 2 Hz, H-9b), 1.54 (1H, dd, 15 and 7 Hz, H-7b), 1.49 (1H, ddd, 12, 8 and 2 Hz, H-11a), 1.27 (1H, t, 12 Hz, H-11b), 1.10 (3H, s, H-12), 1.02 (3H, s, H-13), 0.97 (3H, d, 7 Hz, H-15) and 0.93 (3H, s, H-14).

Synthetic anhydroarthrosporone 56

Arthrosporone **20** (2 mg, 0.008 mmole) was dissolved in dry benzene (3 mL) containing molecular sieves (3 A) and a catalytic amount of *p*-toluenesulfonic acid. The mixture was allowed to stir at room temperature for 4 h. The mixture was decanted, diluted with ether and washed with aqueous sodium bicarbonate (10%, 2 mLx2). The organic extract was dried (Na_2SO_4) and evaporated under reduced pressure. The residue was purified by flash chromatograpy to give a quantitative yield of crystalline, unsaturated ketone **56**.

M.p. 119-120°C.

TLC: R'_f 0.74 (acetone / benzene 3:2), 0.49 (ethyl / acetate / pentane 1:1, developmentx2).

OR: $[\alpha]_D^{23}$ +62.0 (*c* 1.0, $CHCl_3$).

FTIR ($CHCl_3$, cast): 3424 (br), 2951, 2928, 1692 (strong), 1636, 1463, 1376 and 1368 (doublet) cm^{-1}.

HREIMS: m/z (formula, intensity) 234 ($C_{15}H_{22}O_2$, 71.3, $M^{\cdot}$), 219 ($C_{14}H_{19}O_2$, 32.1, $M^{\cdot} - CH_3$), 216 ($C_{15}H_{20}O$, 34.8, $M^{\cdot} - H_2O$), 201 ($C_{14}H_{17}O$, 25.4), 191 ($C_{13}H_{19}O$, 7.8), 178 ($C_{11}H_{14}O_2$, 22.3), 173 ($C_{13}H_{17}$, 26.1), 150 ($C_{10}H_{14}O$, 16.6), 124 ($C_8H_{12}O$, 100.0, $M^{\cdot} - C_7H_{10}O$), 123 ($C_8H_{11}O$, 89.9) 122 ($C_8H_{10}O$, 94.2), 112 ($C_7H_{12}O$, 14.0, $M^{\cdot} - C_8H_{10}O$), 83 (C_5H_7O, 10.3); m/z calcd. for $C_{15}H_{22}O_2$: 234.1614; found: 234.1619.

^1H NMR ($CDCl_3$): δ 5.86 (1H, d, 1.5 Hz), 2.80 (1H, d, 15 Hz), 2.74 (1H, dd, 15.0 and 1.5 Hz), 2.39 (1H, brdd, 11 and 9 Hz), 2.34 (1H, q, 7 Hz), 1.88 (1H, dd, 14 and 2 Hz), 1.73 (1H, ddd, 13, 9 and 2 Hz), 1.70 (1H, d, 14 Hz), 1.49 (1H, dd, 13 and 11 Hz), 1.23 (3H, s), 1.13 (3H, s), 1.11 (3H, d, 7 Hz), 0.94 (3H, s).

cis,anti,cis-2β,3α, 10,10-Tetramethyl-6β,8α- dihydroxytricyclo [6.3.0.0^{2,6}] undecan-4-one (isoarthrosporone, 57)

Arthrosporone **20** (6 mg, 0.024 mmole) was dissolved in dichloromethane containing dimethylaminopyridine (catalytic amount) and diethylamine (4 drops). The mixture was heated under reflux for 24 h, then allowed to cool and diluted with dichloromethane. The dichloromethane was partitioned with water. The organic layer was washed, dried and concentrated to dryness to give a mixture of two products. Silica gel chromatography (acetone/benzene 1:4) provided the starting material **20** and the minor isomer **57**. The starting material was continuously recycled to yield about 2 mg of the desired product, isomer **57** as a waxy material.

TLC: R_f 0.39 (acetone/chloroform 3:7), 0.40 (acetone/benzene 2:5).

OR: $[\alpha]_D^{23}$ +38.2 (*c*, 1.0, $CHCl_3$).

FTIR ($CHCl_3$, cast): 3435 (broad, strong), 1728 (very strong) cm^{-1}.

HREIMS: m/z (formula, intensity) 252 ($C_{15}H_{24}O_3$, 28.3, $M^{\cdot}$), 234 ($C_{15}H_{22}O_2$, 56.6, $M^{\cdot} - H_2O$), 219 ($C_{14}H_{19}O_2$, 32.4, $M^{\cdot} - H_2O - CH_3$), 216 ($C_{15}H_{20}O$, 3.3 $M^{\cdot} - 2H_2O$), 192 ($C_{13}H_{20}O$, 43.7, 234 $-C_2H_2O$), 191 ($C_{13}H_{19}O$, 75.9, 192 – H), 177 ($C_{12}H_{17}O$, 24.2), 163 ($C_{11}H_{15}O$, 43.9), 150 ($C_{11}H_{11}$, 32.3), 125 ($C_7H_9O_2$, 60.2) 109 (C_8H_{13}, 46.1), 83 (C_5H_7O, 100.0).

^{1}H NMR ($CDCl_3$): δ 3.17 (1H, d, 20 Hz, H-5a), 2.46 (1H, dd, 20 and 1.5 Hz, H-5b), 2.29 (1H, qd, 7 and 1.5 Hz, H-3), 2.19 (1H, d, 13 Hz, H-7a), 2.10 (1H, brt, 9.5 Hz, H-1), 1.94 (1H, d, 13 Hz, H-7b), 1.73 (1H, brd, 14 Hz, H-9a), 1.69 (1H, brs, OH), 1.60(1H, ddd, 13, 9 and 1.8 Hz, H-11a), 1.54 (1H, d, 14 Hz, H-9b), 1.47 (1H, dd, 13 and 9 Hz, H-11b), 1.36 (1H, brs, OH), 1.18 (3H, s, H-12), 1.09 (3H, s, H-13), 1.04 (3H, s, H-14), 1.00 (3H, d, 7 Hz, H-15).

^{1}H NMR (C_5D_5N): δ 6.54 (1H, brs, OH), 5.80 (1H, brs, OH), 3.80 (1H, d, 19.5 Hz, H-5a), 2.84 (1H, brd, 19.5 Hz, H-5b), 2.56 (1H, brq, 7.2 Hz, H-3), 2.52 (1H, d, 13.5 Hz, H-7a), 2.44 (1H, brt, 9 Hz, H-1), 2.37 (1H, d, 13 Hz, H-7b), 2.03 (1H, brd, 14 Hz, H-9a), 1.61 (1H, brd, 14 Hz, H-9b), 1.60 (2H, m, H-11), 1.39 (3H, s, H-13), 1.25 (3H, s, H-14), 1.12 (3H, d, 7.2 Hz, H-15), 1.08 (3H, s, H-13).

***cis,anti,cis*-2β,3β, 10,10- Tetramethyl -6β,8α -tricyclo [6.3.0.0^{2,6}] undecan-4-one (diacetoxyarthrosporone diacetate, 58)**

Arthrosporone **20** (2 mg, 0.008 mmole) was dissolved in dichloromethane (2 mL) containing acetic anhydride (5 drops). The solution was allowed to cool in an ice-bath and few grains of *p*-toluenesulfonic acid was added. The monoacetate **27** was formed quantitatively after 30 min as judged by TLC. The mixture was allowed to warm to room temperature. After 60 min the solution was diluted with dichloromethane (10 mL), washed with aqueous 5% sodium bicarbonate (2x 2 mL), brine (2 mL), dried ($MgSO_4$), and evaporated to yield crude diacetate. Purification of the residue by chromatography over silica gel (acetone / dichloromethane 2:98) yielded pure diacetate **58** as a waxy material.

TLC: R'_f 1.14 (acetone / benzene 1:8), 1.08 (ethyl acetate / pentane 1:3).

FTIR ($CHCl_3$, cast): 1739 (strong), 1383 and 1369 (as a doublet), 1249 (strong), 1227 (strong) and 1029 cm^{-1}.

^{1}HNMR ($CDCl_3$): δ 3.31 (1H,d, 17 Hz, H-7a), 3.21 (1H, dd, 19 and 1 Hz, H-5a), 2.86 (1H, dd, 12 and 8 Hz, H-1), 2.31 (1H, d, 15.0 Hz, H-9a), 2.29 (1H, d, 19.0 Hz, H-5b), 2.27 (1H, qd, 7.0 and 1.0 Hz, H-3), 2.18 (1H, d, 17.0 Hz, H-7b), 2.04 (3H, s, CH_3CO-), 1.98 (3H, s, CH_3CO-), 1.67 (1H, t, 12.0 Hz, H-11a), 1.62 (1H, dd, 12 and 8 Hz, H-11b), 1.59 (1H, d,

15.0 Hz, H-9b), 1.06 (3H, s, H-13), 1.05 (3H, d, 7 Hz, H-15), 1.03 (3H, s, H-12), 0.88 (3H, s, H-14).

Attempted epoxidation of anhydroarthrosporone 21

A solution of **21** (4 mg, 0.0169 mmole) in tetrahydrofuran (2 mL), was cooled at 0°C for 15 min, then a solution of sodium bicarbonate (14 mg, 0.169 mmole) in water (2 mL) and 30% hydrogen peroxide (1 drop) was added. The mixture was allowed to stir at room temperature. After 6 h no reaction had taken place as judged by TLC. After 24 h a mixture of starting material and a non-UV active compound (major by TLC) was formed. The reaction was quenched with a saturated solution of ammonium chloride (5 mL), then the mixture was extracted with ethyl acetate (3x10 mL). The organic layers were combined, washed, dried and evaporated *in vacuo*. The residue obtained (4.5 mg) was subjected to a mini silica gel column chromatography (acetone / dichloromethane 3:28) to give mostly the starting material **21** (≈ 2 mg), a solid material **60** (0.8 mg) and a very polar compound **59** which was not identified.

Most polar unidentified compound (59)

TLC: R'_f 0.20 (acetone / benzene 2:7), 0.14 (ethyl acetate / pentane 2:3, developmentx2).

FTIR ($CHCl_3$, cast): 3375 (broad, strong), 2952 (strong), 2932 (strong), 2867, 1736-1713,* 1137 (strong), 1079 (strong) cm^{-1}.

HREIMS: m/z (formula, intensity) 248 ($C_{15}H_{20}O_3$, 6.5, $M^{\cdot} - H_2O$), 205 ($C_{13}H_{17}O_2$, 23.7), 193 ($C_{12}H_{17}O_2$, 85.8), 191 ($C_{13}H_{19}O$, 28.4), 177 ($C_{12}H_{17}O$, 28.2), 176 ($C_{13}H_{16}O$, 61.2), 164 ($C_{11}H_{16}O$, 100.0, $M^{\cdot} - C_4H_8O_2$), 163 ($C_{11}H_{15}O$, 44.9), 161 ($C_{11}H_{13}O$, 28.9), 135 ($C_9H_{11}O$, 37.1), 121 (C_9H_{13}, 52.8), 111 ($C_6H_7O_2$, 30.4), 109 (C_7H_9O, 48.1), 95 (C_7H_{11}, 20.6), 91 (C_7H_7, 43.9), 67 (C_5H_7, 31.9), 55 (C_4H_7, 72.6); m/z calcd. for $C_{15}H_{22}O_4$: 266; found 266 by CI (m/z 284 (M+18) 100.0)).

^1H NMR ($CDCl_3$): δ 4.09 (< 1 H, s), 3.14 (1H, brs, OH), 2.16 (1H, brs, OH), 2.16 (1H, d, 14

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* A medium intensity band indicating that the sample was contaminated with a carbony compound.

Hz), 2.12 (1H, d, 13.5 Hz), 1.94 (1H, brdd, 13 and 7.5 Hz), 1.83 (1H, brd, 12.8 Hz), 1.81 (1H, d, 12.8 Hz), 1.69 (1H, d, 13 Hz), 1.59(1H, dd, 13 and 8 Hz), 1.40 (1H, t, 13 Hz), 1.26, 1.15 1.14 and 0.97 (each 3H, s).

***cis,anti,cis*-2β,3α,10,10-Tetramethyl-8α-hydroxy (3β,6β-epoxytricyclo[6.3.0.0^{2,}]undecan-4-one, 60**

Compound **60** was recrystallized (Et_2O / Skellysolve B) to give needles: m.p. 115-117°C.

TLC: R_f (cholesterol): 0.82 (acetone / benzene 2:7), 0.57 (ethyl acetate / pentane 1:2 developmentx2), as a yellowish spot (reagent A) or reddish spot (reagent B).

FTIR ($CHCl_3$, cast): 3464(broad), 1749 (very strong), 1384, 1180, 1142, 1036 (all strong) cm^{-1}.

HREIMS: m/z (formula, intensity) 207 ($C_{13}H_{19}O_2$, 9.5, $M^+ - (CH_2CO+H)$), 204 ($C_{14}H_{20}O$, 35.8, $M^+ - (H_2O+CO)$), 179 ($C_{12}H_{19}O$, 78.9), 165 ($C_{11}H_{17}O$, 89.8), 164 ($C_{11}H_{16}O$, 52.2), 161 ($C_{12}H_{17}$, 99.6), 149 ($C_{10}H_{13}O$, 21.6), 123 ($C_8H_{11}O$, 55.4), 113 ($C_6H_9O_2$, 45.6), 112 ($C_6H_8O_2$, 32.2), 109 (C_7H_9O, 100), 107 (C_8H_{11}, 30.1), 105 (C_8H_9, 35.8), 95 (C_7H_{11}, 34.4), 91 (C_7H_7, 36.4), 85 (C_5H_9O, 36.6), 81 (C_6H_9, 43.8), 79 (C_6H_7, 45.2), 77 (C_6H_5, 41.0), 55 (C_4H_7, 63.3); m/z calcd. for $C_{13}H_{22}O_3$: 250; found: 250 by CI (m/z 268 (M+18), 100.0)).

^1H NMR ($CDCl_3$): δ 2.78 (1H, d, 18 Hz, H-5a), 2.41 (1H, brdd, 12 and 7 Hz, H-1), 2.40 (1H, dd, 18 and 2 Hz, H-5b), 2.31 (1H, dd, 14 and 2 Hz, H-7a), 2.13 (1H, brd, 14 Hz, H-7b), 1.65 (1H, brs, OH), 1.62 (1H, d, 12 Hz, H-9a), 1.56 (1H, t, 12 Hz, H-11a), 1.51 (1H, d, 12 Hz, H-9b), 1.49 (1H, dd, 12 and 7 Hz, H-11b), 1.18 (3H, s, H-12), 1.15 (3H, s, H-15), 1.4 (3H, s, H-14), 1.06 (3H, H-13).

Oxidation of arthrosporol

Alcohol **22** (3 mg, 0.012 mmole) in dichloromethane (2 mL) was allowed to stir in the presence of pyridinium chlorochromate (PCC, 2.2 mg) at room temperature. After 2 h the suspension was filtered through a florisil column which was then washed with diethyl ether. The organic filtrates were combined and concentrated to yield after recrystallization

(ether / Skellysolve B) keto-diol **63**: m.p. 139-141°C, $[\alpha]_D^{23}$ -145°, (*c* 0.5, $CHCl_3$).

TLC: R'_f 0.51 (acetone / benzene 2:5).

FTIR ($CHCl_3$, cast): 3478 (broad, strong), 2951 (strong), 2868, 1730 (strong), 1382, 1366, 1190, 1141, 1071 cm^{-1}.

HREIMS: m / z (formula, intensity), 252 ($C_{15}H_{24}O_3$, 36.5, M^+), 234 ($C_{15}H_{22}O_2$, 28.8, $M^+ - H_2O$), 216 ($C_{15}H_{20}O$, 2.0, $M^+ - 2H_2O$), 192 ($C_{13}H_{20}O$, 81.4), 191 ($C_{13}H_{19}O$, 29.9), 125 ($C_7H_9O_2$, 100.0; $C_9H_{13}O$, 2.0), 109 (C_8H_{13}, 22.7), 83 (C_5H_7O, 41.2), 69 (C_5H_9, 29.9), 56 (C_4H_7, 25.8).

Triols 64, 65 and 71 obtained from the reduction of arthrosporone 20

Crude **20** (5 mg, 0.02mmole) was dissolved in methanol (2 mL) and sodium borohydride (7 mg, 0.2 mmole) was added. The mixture was allowed to stir at room temperature. After 1 h, aqueous acetic acid (50%, 1 mL) was added and the mixture was allowed to stir for 2 min. The solvents were removed, water (2 mL) was added and the aqueous solution was extracted with ethyl acetate (3x5 mL). The organic layers were combined, washed with brine, dried (Na_2SO_4) and concentrated *in vacuo*. The crude product was purified by chromatography on silica gel (two successive columns, acetone / dichloromethane (3:7) and acetone / benzene (1:4)) to give three products. The most polar product was the major triol while the least polar product was the minor triol.*

***cis,anti,cis*-2β,3β,10,10-Tetramethyltricyclo [6.3.0.0^{2,6}] undecan-4α,6β,8α-triol (synthetic arthrosporol, most polar, 64)**

M.p. 164-165°C.

TLC: R'_f** 0.52 (acetone / benzene 3:2), 0.46 (acetone / benzene 2:3, developmentx2), reddish spot which turned dark blue overnight (reagent A).

]OR: $[\alpha]_D^{23}$ -32.0° (*c* 1.5, $CHCl_3$); -62.3° (*c* 0.7, MeOH).

FTIR ($CHCl_3$, cast): 3368 (broad and intense), 2950, 2927, 1383 cm^{-1}.

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* The third triol whose polarity lies between those of the minor and major triols was not obtained when the reduction was carried out on pure arthrosporone (**20**).

** R'_f was calculated using metabolite **21** for reference.

HREIMS: m/z (fragment, intensity), 236 ($C_{15}H_{24}O_2$, 17.8, $M^{\cdot} - H_2O$), 218 ($C_{15}H_{22}O$, 100.0, $M^{\cdot} - 2H_2O$), 203 ($C_{14}H_{19}O$, 24.6, 218-CH_3), 182 ($C_{11}H_{18}O_2$, 32.7, $M^{\cdot} - C_4H_8O$), 114 ($C_{13}H_{18}$, 25.6), 150 ($C_{11}H_{18}$, 39.8), 139 ($C_9H_{15}O$, 19.5), 127 ($C_7H_{11}O_2$, 19.0), 109 (C_8H_{13}, 21.6), 83 (C_5H_7O, 16.6), 55 (C_4H_7, 12.2).

CIMS: m/z (intensity,f ragment) 272 (100.0, $M{\cdot}NH_4^+$), 254 (70.4, $M{\cdot}NH_4^+ - 18$), 236 (22.8, $M^{\cdot} - H_2O$).

^{1}H NMR ($CDCl_3$): δ 3.94 (1H, td, 9 and 5 Hz, H-4), 2.38 (1H, brdd, 13 and 8 Hz, H-1), 2.31 (1H, d, 15 Hz, H-7a), 2.21 (1H, d, 15 Hz, H-7b), 2.04-2.00 (3H, m), 1.87 (1H, brd, 14 Hz, H-9a), 1.71 (1H, brd, 14 Hz, H-9b), 1.54 (1H, brt, 12 Hz, H-11a) 1.47 (1H, brddd, 12, 9 and 2.2 Hz, H-11b), 1.11 (3H, s, H-12), 1.04 (3H, s, H-13), 1.02 (3H, d, 7 Hz, H-15), 0.78 (3H, s, H-14).

***cis,anti,cis*2β,3β,10,10- Tetramethyltricyclo [6.3.0.0^{2,6}]undecan-4β,6β,8α-triol (*epi*-arthrosporol, least polar triol 65)**

M.p. 150°C (dec.) (diethyl ether / Skellysolve B).

TLC: R'_f 0.63 (acetone / benzene 3:2), 0.55 (acetone / dichloromethane, 3:2, developmentx2), reddish then blue spot.

OR: $[\alpha]_D^{23}$ -64.0° (*c* 0.1, MeOH).

FTIR ($CHCl_3$, cast): 3301 (broad, strong), 2950, 2930, 1466, 1378 and 1364 (doublet), 1067, 1024 cm^{-1}.

HREIMS: m/z (formula, intensity) 254 ($C_{15}H_{26}O_3$ 3.3, $M^{\cdot}$), 236 ($C_{15}H_{24}O_2$, 19.8, $M^{\cdot} - H_2O$), 218 ($C_{15}H_{22}O$, 7.5, $M^{\cdot} - 2H_2O$), 203 ($C_{14}H_{19}O$, 218-CH_3), 182 ($C_{11}H_{18}O_2$, 100.0,$M^{\cdot} - C_4H_8O$), 177 ($C_{12}H_{17}O$, 4.0), 174 ($C_{13}H_{18}$, 21.2), 164 ($C_{11}H_{16}O$, 6.8), 150, ($C_{11}H_{18}$, 38.5), 139 ($C_9H_{15}O$, 42.0), 127 ($C_7H_{11}O_2$, 17.5 $M^{\cdot} - C_8H_{14}O$), 124 ($C_8H_{12}O$, 3.6), 121 (C_9H_{13}, 22.4), 111 ($C_7H_{11}O$, 5.4), 83 (C_5H_7O, 14.0), 55 (C_4H_7, 26.6); m/z calcd for $C_{15}H_{24}O_3$: 254.1875; found: 254.1875.

^{1}H NMR ($CDCl_3$): δ 4.14 (1H, td, 5.0 and 2.0 Hz), 2.51 (1H, dd, 14.1 and 5.0 Hz), 2.31 (1H, td, 11.0 and 1.5 Hz), 2.13 (1H, dd, 14.0 and 1.5 Hz), 1.07 (1H, dq, 5.0 and 7.2 Hz), 1.98 (1H, d, 14.0), 1.87 (1H, brd, 13.8 Hz), 1.85 (1H, dd, 14.1 and 1.8 Hz), 1.57 (1H, brd, 13.5

Hz), 1.48 (2H, M), 1.09 (3H, s), 1.03 (3H, s), 1.00 (3H, d, 7.2 Hz), 0.92 (3H, s), 2.07 and 1.57 (2H exchangeable with D_2O).

***cis,anti,cis*-2β,3α,10,10-Tetramethyltricyclo[6.3.0.0^{2,6}] undecan-4β,6β,8α-triol (*iso*-arthrosporol, 71)**

M.p. 168-169°C (CH_2Cl_2 / Skellysolve B).

TLC: R_f 0.52 (acetone / benzene 3:2); 0.42 (acetone / dichloromethane 3:2 developmentx2).

OR: $[\alpha]_D^{23}$ -20.9° (*c* 2.2, MeOH).

FTIR ($CHCl_3$, cast): 3301 (broad and intense), 2950, 2980, 2803, 1466, 1378 and 1364 (doublet), 1315, 1163, 1067.

HREIMS: m / z (formula, intensity) 236 ($C_{15}H_{24}O_2$, (93.8,$M^+ - H_2O$), 218 ($C_{15}H_{22}O$, 100.0, 236-H_2O), 203 ($C_{14}H_{19}O$, 67.2, 218-CH_3), 182 ($C_{11}H_{18}O_2$, 36.1, $M^+ - C_4H_8O$), 177 ($C_{12}H_{17}O$, 16.0), 175 ($C_{13}H_{19}$, 23.0), 164 ($C_{11}H_{16}O$, 39.7), 150 ($C_{11}H_{18}$, 35.0), 139 ($C_9H_{15}O$, 30.6), 127 ($C_7H_{11}O_2$, 9.2, $M^+ - C_8H_{13}O$), 124 ($C_8H_{12}O$, 3.3, $M^+ - C_7H_{14}O_2$), 121 (C_9H_{13}, 25.6), 111 ($C_7H_{11}O$, 27.2), and 83 (C_5H_7O, 42.1).

^1H NMR ($CDCl_3$): δ 4.16 (1H, brt, 6.5 Hz, H-4), 2.90 (1H, ddd, 13.75 and 1.5 Hz, H-1), 2.47 (1H, brd, 16 Hz, H-5a), 2.37 (1H, dd, 14 and 1.7 Hz, H-7a), 2.27 (1H, brdd, 16 and 6.5 Hz, H-5b), 1.86 (1H, d, 14 Hz, H-7b), 1.84 (1H, m, H-3), 1.68 (1H, dd, 14 and 2.5 Hz, H-9a), 1.51 (1H, d, 14 Hz, H-9b), 1.46 (1H, ddd, 12, 8 and 2.5 Hz, H-11a), 1.41 (1H, brt, 11 Hz, H-11b), 1.12 (3H, s, H-12), 1.08 (3H, d, 7 Hz, H-15), 1.03 (3H, s, H-13), 0.89 (3H, s, H-14).

***cis,anti,cis*-2β,3β,10,10-Tetramethyl-4α-acetoxytricyclo [6.3.0.0^{2,6}] undecan-6β,8α-diol (4-O-acetyl arthrosporol, 66)**

A solution of arthrosporol (3.4 mg, 0.013 mmole), acetic anhydride (5 drops) and pyridine (1.5 mL) was allowed to stir at room temperature for 24 h. Workup in the usual manner yielded crude acetate (4 mg). Purification by silica gel chromatography (acetone / dichloromethane 12:88) gave monoacetate 66 which was recrystallized

(Skellysolve B / diethyl ether): m.p. 150°C (dec.).

TLC: R'_f 0.71 (acetone / benzene 2:7), 0.35 (ethyl acetate / pentane 1:2).

OR: $[\alpha]_D^{23}$ -58.4 (*c* 2, $CHCl_3$).

FTIR ($CHCl_3$, cast): 3456 (broad), 2951 (strong, 2936 (strong), 2865, 1732 (strong), 1715 (strong), 1260 (strong) 1020 cm^{-1}.

LREIMS: m / z (intensity), 296 (0.1), 219 (18.0), 218 (100.0), 203 (14.4), 160 (11.5), 136 (42.3), 125 (12.2), 109 (39.9), 95 (15.5), 83 (18.4), 69 (17.1), 67 (10.6), 55 (26.6), 43 (79.9).

HREIMS: m / z (formula, intensity) 278 ($C_{17}H_{26}O_3$, 0.2, $M^+ - H_2O$), 236 ($C_{15}H_{24}O_2$, 3.5 ($M^+ - AcOH$), 218 ($C_{15}H_{22}O$, 100.0, $M^+ - AcOH - H_2O$), 203 ($C_{14}H_{19}O$, 17.8), 136 ($C_{10}H_{16}$, 41.1; $C_9H_{12}O$, 9.8), 123 ($C_8H_{11}O$, 11.2), 110 ($C_7H_{10}O$, 25.0), 109 (C_8H_{13}, 22.4; C_7H_9O, 26.5), 55 (C_4H_7, 23.8).

^1H NMR ($CDCl_3$): δ 4.84 (1H, ddd, 9.5, 9 and 4 Hz, H-4), 2.42 (1H, brdd, 12 and 8 Hz, H-1), 2.27 (1H, brd, 16 Hz, H-7a), 2.22 (1H, d, 16 Hz, H-7b), 2.22 (1H, dq, 9 and 7 Hz, H-3), 2.13 (1H, dd, 15 and 9 Hz, H-5a), 2.03 (3H, s, CH_3CO), 1.95 (1H, dd, 15 and 4 Hz, H-5b), 1.90 (1H, d, 14 Hz, H-9a), 1.71 (1H, dd, 14 and 2.5 Hz, H-9b), 1.63 (1H, brs, OH), 1.59 (1H, t, 12 Hz, H-11a), 1.40 (1H, ddd, 12, 8 and 2.5 Hz, H-11b), 1.12 (3H, s, H-1), 1.05 (3H, s, H-13), 0.98 (3H, d, 7 Hz, H-15), 0.79 (3H, s, H-14).

cis,anti,cis-2β,3β,10,10-Tetramethyl-4α,8α- diacetoxytricyclo [6.3.0.0^{2,6}] undecan-6β-ol (arthrosporol diacetate, 67)

Arthrosporol (22, 4.7 mg, 0.019 mmole), dimethylaminopyridine (catalytic amount), and acetic anhyhydride (5 drops) were dissolved in triethylamine at room temperature. The reaction mixture was allowed to stand for 3 days. The mixture was then worked up in the usual way. The crude acetate was purified by chromatography (acetone / benzene 5:95) to give diacetate 67 as a waxy material.

TLC: R'_f 0.86 (acetone / benzene 7:2), 0.78 (ethyl acetate / pentane 2:1).

FTIR ($CHCl_3$, cast): 3512 (broad), 2952, 1732 (strong), 1717 (shoulder), 1249 (strong), 1020 (strong) cm^{-1}.

FTIR: ($CHCl_3$, 0.1 MM) 3560, 1716 (strong), 1248 (strong) cm^{-1}

FTIR (CCl_4, 0.1 MM): 3615, 2958, 1734 (strong), 1242 (strong).

LREIMS: m/z (relative intensity) 299 (0.6), 278 (2.7), 219 (50.4), 218(100.0), 203 (12.8), 190 (16.6), 163 (23.3), 109 (30.1), 85 (16.5), 55 (13.6), 43 (58.1).

^1H NMR ($CDCl_3$): δ 4.82 (1H, ddd, 10, 9 and 4 Hz,H-4), 2.70 (1H, d, 12 and 9 Hz, H-1), 2.64 (1H, d, 15 Hz, H-7a), 2.34 (1H, brd, 15 Hz, H-7b), 2.20 (1H, dd, 15 and 3 Hz, H-9a), 2.13 (1H, brdd, 14.5 and 10 Hz, H-5a), 2.04, (3H, s, CH_3CO), 2.03 (1H, m, H-3), 2.02 (3H, s, CH_3CO), 1.88 (1H, dd, 14.5 and 4 Hz, H-5b), 1.82 (1H, d, 15 Hz, H-9b), 1.59 (1H, t, 12 Hz, H-11a), 1.44 (1H, dd, 12, 8 and 3 Hz, H-11b), 1.03 (3H, s, H-12), 1.01 (3H, s, H-13), 1.00 (3H, d, 6.5 Hz, H-15), 0.81 (3H, s, H-14);

(C_5D_5N): δ 6.23 (1H, s, OH), 5.12 (1H, td, 9.5 and 4 Hz, H-4), 3.08 (1H, d, 15.5 Hz, H-7a), 2.91 (1H, dd, 12 and 8 Hz, H-1), 2.68 (1H, d, 15 Hz, H-5a), 2.52 (1H, dd, 14 and 10Hz, H-5a), 2.45 (1H, dd, 15 and 2.5 Hz, H-9a), 2.35 (1H, dq, 8.5 and 7 Hz, H-3), 2.19 (1H, brd, 14.5 Hz, H-9b), 2.08 (1H, dd, 14 and 4 Hz, H-5b), 1.96 (1H, brt, 12 Hz, H-11a), 1.96(3H, s, CH_3CO), 1.95 (3H, s, CH_3CO), 1.50 (1H, ddd, 12, 8 and 2.5 Hz, H-11b), 1.09 (3H, s, H-12), 1.09 (3H, d, 7 Hz, H-15), 1.04 (3H, s, H-13), 1.02 (3H, s, H-14).

cis,anti,cis-2β,3β,10,10-Tetramethyl- 4α,6β8α- triacetoxytricyclo [6.3.0.$0^{2,6}$] undecane (arthrosporol triacetate, 68)

Alcohol **22** (2.4 mg, 0.009 mmole) and _p_-toluenesulfonic acid (few grains) were dissolved in acetic anhydride (1 mL) and the solution was allowed to stir at room temperature for 3 h. The solution was diluted with diethyl ether (10 mL), washed (5% aqueous sodium bicarbonate, water and brine), dried ($MgSO_4$) and the solvent removed. The residue was chromatographed over silica gel and pure arthrosporol triacetate (**68**) was eluted (acetone/dichloromethane 1:99) as a viscous material: $[\alpha]_D^{23}$ -29.6 (_c_ 2.1 $CHCl_3$).

TLC: R'_f 1.43 (acetone/benzene 1:8), 1.17 (ethyl acetate/pentane 1:3), developmentx2).

FTIR ($CHCl_3$, cast): 1738 (strong, sharp), 1249 (strong), 1225 (strong), 1020 cm^{-1}.

HREIMS: m/z (formula, intensity) 321 ($C_{17}H_{29}O_4$, 1.1, M^+ – AcO), 260 ($C_{17}H_{24}O_2$, 10.8 (M^+ – 2AcOH), 218 ($C_{15}H_{22}O$, 8.7, M^+ – AcOH – CH_2CO), 200 ($C_{15}H_{20}$, 100.0, M^+ – 3AcOH).

CIMS (NH_3): m/z calcd for M+18: 398; found 398 (100.0%).

^{1}H NMR ($CDCl_3$): δ 4.85 (1H, td, 10 and 3.8 Hz, H-4), 3.19 (1H, d, 17 Hz, H-7a), 2.75 (1H, dd, 12 and 9 Hz, H-1), 2.43 (1H, dd, 15 and 3.8 Hz, H-5a), 2.32 (1H, d, 17 Hz, H-7b), 2.30 (1H, dd, 15 and 10 Hz, H-5b), 2.25 (1H, dd, 15 and 2.8 Hz, H-9a), 2.06, 2.03 and 2.00 (each 3H, s, CH_3CO), 2.02 (1H, m, H-3), 1.58 (1H, brt, 12 Hz, H-11a), 1.53 (1H, brd, 15 Hz, H-9b), 1.49 (1H, ddd, 12, 9 and 2.8 Hz, H-11b), 1.01 (3H, s, H-12), 1.00 (3H, s, H-13), 0.99 (3H, d, 7 Hz, H-15), 0.84 (3H, s, H-14).

***cis*-2β,3α,10,10-Tetramethyl-9α-acetoxytricyclo[6.3.0.0^{2,6}] undec-1(8)-en-4,11-dione (allylic acetate, 72)**

Alcohol **23** (4 mg, 0.016 mmole), pyridine (1.5 mL) and acetic anhydride (few drops) were allowed to stir at room temperature for 12 h. The reaction mixture was worked up in the usual way, then purified by preparative thin layer chromatography (acetone/dichloromethane 5:95) to give pure acetate **72** (3 mg).

TLC: R'_f 1.07 (acetone/benzene 1:8), 0.58 (ethyl acetate, pentane 1:3, developmentx2).

OR: $[\alpha]_D^{23}$ -13.8 (*c* 1.6, $CHCl_3$).

FTIR ($CHCl_3$, cast): 1740 (strong), 1710 (strong), 1632, 1328 and 1374 (doublet), and 1232 (strong) cm^{-1}.

HREIMS: m/z (formula, intensity) 290 ($C_{17}H_{22}O_4$, 6.7, M^+), 248 ($C_{15}H_{20}O_3$, 88.7 (M^+ – CH_3CO), 233 ($C_{14}H_{17}O_3$, 100.0, 248 – CH_3), 177 ($C_{11}H_{12}O_2$, 25.4), 174 ($C_{12}H_{14}O$, 19.2) and 91 (C_7H_7, 18.9); m/z calcd. for $C_{17}H_{22}O_4$: 290.1512; found: 290.1524.

CIMS (NH_3): m/z (relative intensity) 598 (0.3, 2M+18) 308 (100.0 M+18).

^{1}H NMR ($CDCl_3$): δ 5.60 (1H, t, 2.5 Hz, H-9), 2.97 (1H, m, H-6), 2.87 (1H, ddd, 16, 9 and 2.4 Hz, H-7a); 2.59 (1H, dd, 20 and 9 Hz, H-5a), 2.42 (1H, ddd, 19, 4, and 1.5 Hz, H-5b), 2.40 (1H, qd, 7 and 1.5 Hz, H-3), 2.25 (1H, ddd, 16, 7 and 2.5 Hz, H-7b), 2.17 (3H, s, CH_3CO), 1.26 (3H, s, CH_3), 1.11 (3H, s, CH_3), 1.08 (3H, d, 7 Hz, H-15), 1.00 (3H, s, CH_3).

^{13}C NMR (100 MHz, $CDCl_3$): δ 217.5 (s, C-4), 204.9 (s, C-11) 181.4 (s, C-3), 170.3 (s, $CH_3\underline{C}O$), 147.5 (s, C-1), 75.5 (d, C-9), 53.9 (s, C-10), 51.0 (d, C-3), 49.9 (d, C-6), 42.1 (s, C-2), 41.7 (t, C-5), 31.7 (t, C-7), 24.1 (q), 20.7 (q), 19.9 (q, CH_3CO), 18.6 (q), and 9.4 (q).

cis-2β-3α,10,10-Tetramethyltricyclo [6.3.0.0^{2,6}] undec-1(8)- en -4, 9, 11-trione (triketone, 73):

Alcohol **23** (2.7 mg, 0.011 mole) was dissolved in dichloromethane, pyridinium chlorochromate (2.3 mg, 0.013 mmole) was added and the yellow suspension was allowed to stir at room temperature. After 20 h, the brown suspension was filtered through fluorosil and the adsorbent washed with chloroform. Purification of the residue by silica gel chromatography (dichloromethane) gave triketone **73** as a low melting solid.

TLC: R'_f 1.29 (acetone / benzene 1:8), 0.67 (ethyl acetate / pentane 1:3, developmentx2).*

OR $[\alpha]_D^{23}$ -153.9° (_c_ 0.7, $CHCl_3$).

UV (MeOH): λ_{max} 247 (ε 4762); ;236 (ε 41.94).

FTIR ($CHCl_3$, cast): 1736 (strong), 1696 (strong), 1616 (weak) cm^{-1}.

HREIMS: m / z (formula, intensity) 246 ($C_{15}H_{18}O_3$, 100.0, M^+), 231 ($C_{14}H_{15}O_3$, 6.7, $M^+ - CH_3$), 218 ($C_{14}H_{18}O_2$, 2.6, $M^+ - CO$), 190 ($C_{12}H_{14}O_2$, 29.2), 189 ($C_{12}H_{13}O_2$, 53.3), 176 ($C_{11}H_{12}O_2$, 50.1); m / z calcd. for $C_{15}H_{18}O_3$: 246.1251; obs. 246.1257.

^1H NMR ($CDCl_3$): δ 3.10 (1H, dd, 17 and 9 Hz, H-7a), 3.07 (1H, m, H-6), 2.74 (1H, dd, 18.5 and 9 Hz, H-5a), 2.51 (1H, dd, 17 and 6 Hz, H-7b), 2.39 (1H, m, H-3), 2.21 (1H, dd, 18.5 and 5 Hz, H-5b), 1.23 (3H, s, CH_3), 1.19 (3H, s, CH_3), 1.16 (3H, s, CH_3), 1.15 (3H, d, 7 Hz, H-15).

cis,2β,10,10-Trimethyl-11-acetoxy-(1α, 3α)- epoxymethanotricyclo [6.3.0.0^{2,6}] undec-5-en-4-one (O-acetyltetracyclic ether 76)

About 1 mg of **25** was dissolved in pyridine (1 mL) and acetic anhydride (2 drops) was added. The mixture was allowed to stir at room temperature. After 24 h the starting material was recovered unchanged. The solution was evaporated to dryness and the residue redissolved in triethylamine (1 mL). Acetic anhydride (2 drops) and few grains of

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* The compound does not char with a staining reagent A.

DMAP were added and the mixture was allowed to stir overnight. Workup in the usual way gave a complex mixture (TLC). Chromatography of the mixture on a mini silica gel column ($CHCl_3$) gave a small amount of UV-active acetate **76**. IR ($CHCl_3$, cast): 1744 (strong), 1709 (strong), 1632 to 1233 (strong) cm^{-1}.

^1H NMR ($CDCl_3$): δ 5.88 (1H, d, 2 Hz, H-5), 5.09 (1H, s, H-11), 3.97 (1H, dd, 7 and 4 Hz, H-15a), 3.52 (1H, t, 8 Hz, H-8), 3.51 (1H, t, 7 Hz, H-15b), 3.03 (1H, dd, 15 and 9 Hz, H-7a), 2.84 (1H, ddd, 15, 8.5, 15 Hz, H-7b), 2.78 (1H, dd, 7 and 4 Hz), 2.20 (3H, s, CH_3CO), 1.32 (3H, s, H-13), 1.27 (3H, s, H-12), 1.14 (3H, s, H-14).

***trans,cis*,4α,8β,11,11-Tetramethyl-5β-acetoxytricyclo [7.2.0.0^{2,4}] undecan-5β-ol (5-O-acetylisotricyclohumuladiol, 79)**

Alcohol **26** (2 mg, 0.008 mmole) was dissolved in dry pyridine (1.5 mL) and acetic anhydride (3 drops) was added. The mixture was allowed to stir at room temperature for 18 h. Solvents were removed *in vacuo* and the residue was dissolved in dichloromethane. The solution was washed, dried and concentrated to dryness. Purification by chromatography over a silica gel column (chloroform) gave acetate **79**.

TLC: R'_f 0.45 (acetone / chloroform 15:85).

FTIR ($CHCl_3$, cast): 3440 (broad), 2953, 2933, 1731 (strong), 1248 (strong), 1075, 1020, 968, 875 cm^{-1}.

HREIMS: m/z (formula, intensity) 280 ($C_{17}H_{28}O_3$, 2.7, M^+), 263 ($C_{17}H_{27}O_2$, 2.7, $M^+ - H_2O$), 220 ($C_{13}H_{24}O$, 71.6, $M^+ - HOAc$), 187 ($C_{14}H_{19}$, 27.3), 164 ($C_{11}H_{16}O$, 34.7, $M^+ - HOAc - C_4H_8$),, 162 ($C_{12}H_{18}$, 43.1), 159 ($C_{12}H_{15}$, 29.3), 14.9 ($C_{11}H_{17}$, 16.2; $C_{10}H_{13}O$, 44.7), 147 ($C_{11}H_{15}$, 56.5), 146 ($C_{11}H_{14}$, 60.3), 138 ($C_9H_{14}O$, 25.9), 135 ($C_{10}H_{15}$, 26.2), 133 ($C_{10}H_{13}$, 27.2), 131 ($C_{10}H_{11}$, 56.8), 125 ($C_8H_{13}O$, 25.2), 121 (C_9H_{13}, 46.8), 119 (C_9H_{11}, 38.3), 109 (C_8H_{13}, 32.2), 107 (C_8H_{11}, 52.79), 106 (C_8H_{10}, 100.0), 105 (C_8H_9, 34.1), 95 (C_7H_{11}, 58.9), 94 (C_7H_{10}, 51.2), 93 (C_7H_9, 71.6), 91 (C_7H_7, 47.9), 81 (C_6H_9, 41.2), 79(C_6H_7, 37.6), 71 (C_4H_7O, 29.2), 69 (C_5H_9, 32.4), 67 (C_5H_7, 29.9), 59 (C_3H_7O, 28.5) and 55 (C_4H_7, 45.9): m/z calcd. for $C_{17}H_{28}O_3$: 280.2031; found 280.2043.

^1H NMR ($CDCl_3$ + D_2O (1 drop)): δ 4.50 (1H, distorted dd, 12 and 5 Hz, H-5), 2.06 (3H,

CH_3CO), 1.98 (1H, td, 11 and 8 Hz, H-9), 1.84 (3H, m, H-6a and H-7), 1.68 (1H, m, H-6b), 1.56 (1H, dd, 10.5 and 8 Hz, H-10a), 1.42 (1H, t, 11 Hz, H-10b), 1.19 (1H, t, 11 Hz, 11 Hz, H-1),* 1.19 (3H, s, H-15), 1.12 (3H, s, H-12 or H-13), 1.05 (6H, s, H-14 and H-13 or H-12), 0.65 (1H, ddd, 11, 7.5 and 5.5 Hz, H-2),** 0.45 (1H, dd, 7.5 and 5.5 Hz, H-3α), 0.31 (1H, t, 5.5 Hz, H-3β).

trans,cis,4α,8β,11,11-Tetramethyl-11α-hydroxytricyclo [7.2.0.0^{2,4}] undecan-5-one (ketoalcohol 80)

Compound **26** (2 mg, 0.008 mmole) in dichloromethane was oxidized with an excess of pyridinium chlorochromate. After 20 h the mixture was worked up in the usual way to give 2.8 mg of crude ketol: Purification by chromatography over silica gel (chloroform) gave pure ketol **80**.

TLC: R'_f 0.75 (acetone / chloroform 3:7).

OR: $[\alpha]_D^{23}$ -117° (_c_ 1.0, $CHCl_3$).

UV (MeOH): λ_{max} 203 nm (ε 1676).

FTIR ($CHCl_3$, cast): 3427 (broad), 2955, 2932, 1690 (strong), 1384 and 1367 (doublet), 1102, 1089, 1073, 946, 891 cm^{-1}.

HREIMS: m / z (fragment, intensity) 236 ($C_{15}H_{24}O_2$, 36.0, M^+), 221 ($C_{14}H_{21}O_2$, 18.5, $M^+ - CH_3$), 218 ($C_{15}H_{22}O$, 17.1, $M^+ - H_2O$), 179 ($C_{12}H_{19}O$, 20.8), 178 ($C_{12}H_{18}O$, 21.9), 165 ($C_{11}H_{17}O$, 27.2), 163 ($C_{12}H_{19}$, 21.0), 141 ($C_9H_{13}H_{21}$, 31.7), 137 ($C_{10}H_{17}$, 40.3; $C_9H_{13}O$, 21.7), 135 ($C_{10}H_{15}$, 27.7; $C_9H_{11}O$, 24.9), 123 ($C_8H_{11}O$, 36.3), 122 (C_9H_{14}, 40.8; $C_8H_{10}O$, 20.4), 121 (C_9H_{13}, 25.7), 119 (C_9H_{11}, 31.2), 111 ($C_7H_{11}O$, 36.5), 109 (C_8H_{13}, 43.9; C_7H_9O, 57.7), 107 (C_8H_{11}, 56.4), 99 ($C_5H_7O_2$, 100.0), 97 (C_6H_9O, 32.2), 95 (C_7H_{11}, 48.6), 93 (C_7H_9, 52.5), 91 (C_7H_7, 31.6), 79 (C_6H_7, 35.2), 71 (C_4H_7O, 25.1), 69 (C_4H_5O, 23.8; C_5H_9, 68.5), 67 (C_5H_7, 56.7), 57 (C_4H_9, 57.4) and 55 (C_4H_7, 91.7); m / z calcd. for $C_{15}H_{24}O_2$: 236.1770; found 236.1777.

^1H NMR ($CDCl_3$ + D_2O (1 drop)): δ 2.79 (1H, ddd, 13.5, 5.3 and 3.5 Hz, H-6a), 2.42 (1H, td,

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* The center of the triplet coincides with the methyl singlet at δ 1.19.
** Coupling constants of the cyclopropane protons: J_{cis} = 7.5 Hz, J_{trans} = J_{gem} = 5.5 Hz.

13.5 and 4 Hz, H-6b), 2.34 (1H, td, 13.5 and 4 Hz, H-7a), 2.04 (1H, td, 10 and 8 Hz, H-9), 1.91 (1H, ddd, 13.5, 5.1 and 3.4 Hz, H-7b), 1.54 (1H, dd, 10 and 8 Hz, H-10a), 1.38 (3H, s, H-15), 1.36 (1H, t, 11Hz, H-10b), 1.19 (1H, t, 5.5 Hz), 1.09 (3H, s, H-12), 1.06 (3H, s, H-13 or H-14), 0.99 - 0.91 (1H, m) 0.96 (3H, s, H-14 or H-13), 0.52 (1H, dd, 8 and 5.5 Hz).

STUDY OF THE BIOLOGICAL ACTIVITY OF ARTHROSPORAE METABOLITES USING A PAPER DISK DIFFUSION METHOD (modified Kirby-Bauer method):

Preparation of the tested cultures

A solution of Bactrol Disk* was aseptically prepared by allowing a disk of bacteria in 2 mL Mueller Hinton (Difco bacto) broth to incubate overnight at 35-37°C. Mueller-Hinton agar plates were then swabbed by lightly brushing the plate surface with the above inoculum using a Q-tip swab.

Inoculum of test fungi were prepared in a manner similar to that described above. Potato dextrose broth and potato dextrose agar were used as culture media (time varied with species overnight to several days).

Test solution and results

Compounds to be tested were prepared as follows. A solution of the compound (or mixture) was prepared at concentrations of 5% (crude material) or 2 to 5% (pure material). Paper disks (10 or 6 mm in diameter) were soaked in the solution, then allowed to dry. Agar plates were inoculated with bacteria, the compound soaked disks were firmly placed on agar and the plates were incubated for 24 h (bacteria) or for several days (slow-growing fungal test cultures).** The results of antibiotic assays of Arthrosporae metabolites are shown in Tables 3 - 7.

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*Bactrol Disks are water soluble disks containing viable bacteria commercially available from American Type Culture Collection (ATCC).

** For detailed procedure see Laboratory Manual by L. Browne and A. Szenthe, ed. 1985, Department of Chemistry, University of Alberta.

Table 3. Antibiotic Bioassay of Crude Extracts of Arthrosporae

Test Microorganisms	Zone diameters** of inhibition E-1*	E-2	E-3	E-4
Enterobacter cloacae	12	14	–	–
Escherichia coli	–	14	–	–
Proteus vulgaris	30	30	20	–
Serratia marcescens	20	20	–	–
Staphylococcus aureus	22	24	–	–
Staphylococcus epidermidis	18	20	–	–
Streptococcus pyogenes		Very positive	–	–
Candida albicans	28	18		

**A 10 mm disk was soaked with 5% solution of the material to be tested.

* E-1: concentrated ether extract from the culture broth.

E-2: concentrated ethyl acetate extract from the culture broth.

E-3: concentrated ether extract from mycelium.

E-4: concentrated ethyl acetate extract from mycelium.

Note – means no activity.

Table 4: Antibiotic Bioassay of Crude Extracts from Arthrosporae

	Zone diameters** of inhibition			
Test Microorganisms	E-5*	E-6	E-7	E-8
Serratia marcescens	–	–	25	–
Staphylococcus aureaus	12	10	18	10
Staphylococcus epidermidis	18	11	16	–
Streptococcus pyogenes	20	20	22	–
Candida albicans	15	8	18	–

*E-5 (neutral extract), E-6 (acidic extract), E-7 (crude extract, and E-8 (basic extract of the culture broth)

** A 6 mm disk was soaked with a 5% solution (CH_2Cl_2 / MeOH, 95 / 5) of extract.

Table 5: Antifungal Bioassay of Crude Extracts of Arthrosporae

Test Fungi *(Ceratocystis)*	Zone diameters (mm*) of inhibition			
	E-9**	E-10	E-11	E-12
C. clavigera	35	36	20	22
C. minor (C-248)	24	32	–	17
C. minor (C-839)	24	30	–	24
C. ulmi	20	22	18	16

* A 10 mm disk was soaked in a 5% solution of the extract.

** E-9 (total crude extract), E-10 (neutral extract), E-11 (acidic extract) from still culture broth, and E-12 (ether extract) of fermentor broth.

Table 6: Antifungal Bioassay of Pure Metabolites

	Metabolites			
Test Fungi	**20**	**21**	**22**	**23**
Ceratocystis clavigera	14*	14	b	12
Ceratocystis minor (C-839)	a	a	a	–
Ceratocystis montia	28	30	30	18
Ceratocystis ulmi	7	8	–	–
Verticillium wagneria	123	18	a	8

* Zone diameters (mm) of Inhibition

a. slight activity.

b. inhibition zone not well defined for measurement.

Antifungal activity of anhydroarthrosporone (**21**) and dehydroarthrosporodione (**23**) was compared with Arbotect (Merck) (**83**) and Lignasan BLP (Dupont) (**84**),* two well-known benzimidazole derivatives used as fungicides against plant pathogen fungi including the *Ceratocystis*. Results of the antifungal bioassay are presented in Table 7.

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* Samples of Arbotect (**83**) and Lignasan (**84**) were kindly provided by Dr Y. Hiratsuku, Northern Forest Research Center, Canadian Forestry Service.

Table 7: Antifungal Bioassay of Known Fungicides

	Fungicides*			
	21	**23**	**83**	**84**
Test Fungi (*Ceratocystis*)				
C. minor	8**	10	30	12
C. ulmi	8	10	30	–

* The concentrations of each of **83** and **84** was 0.1%, **21** was 4% and **23** was 2%.

** Zone diameters(mm) of inhibition (6 mm diameter disk was used).

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APPENDIX

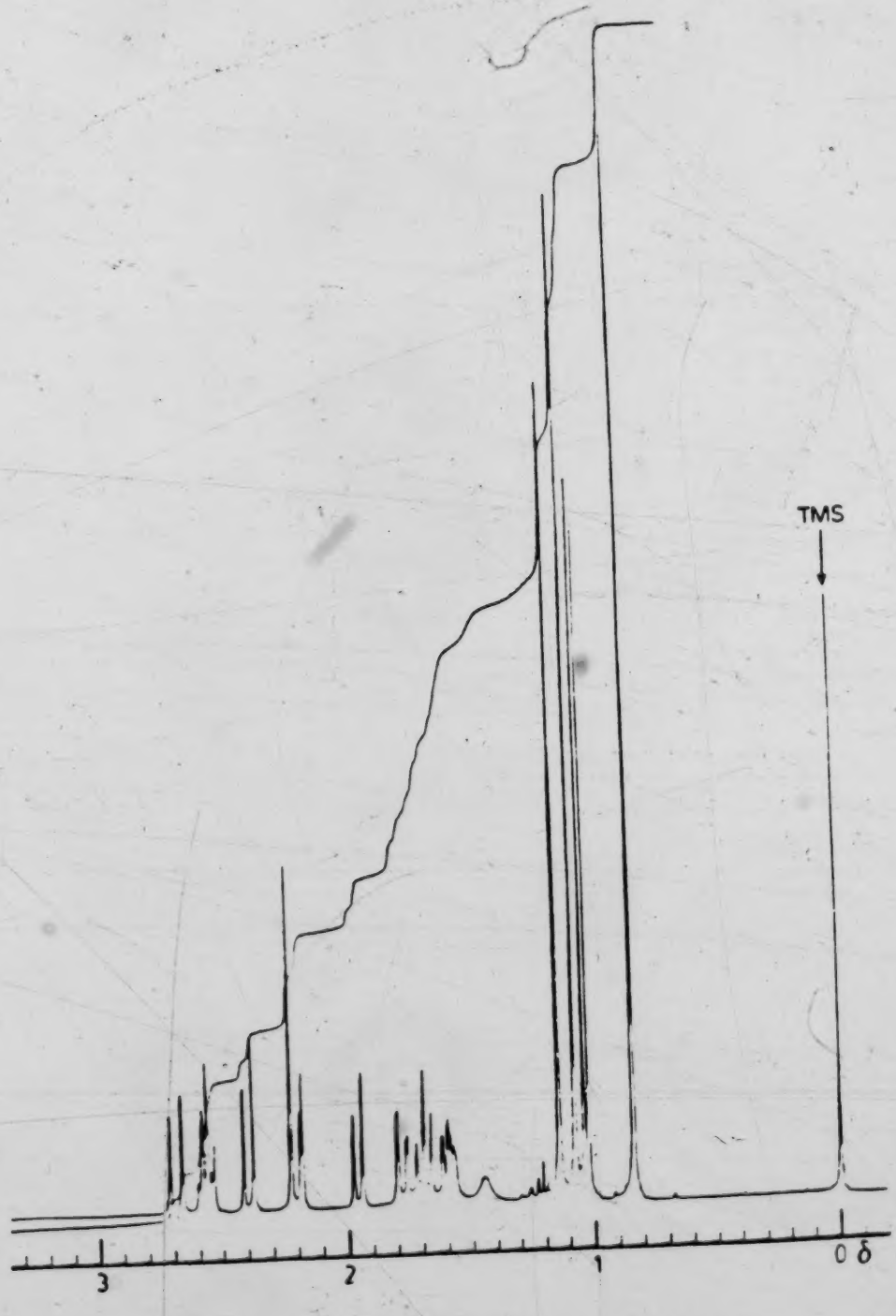

Figure 2. 400 MHz ^{1}H NMR spectrum ($CDCl_3$) of arthrosporone (20)

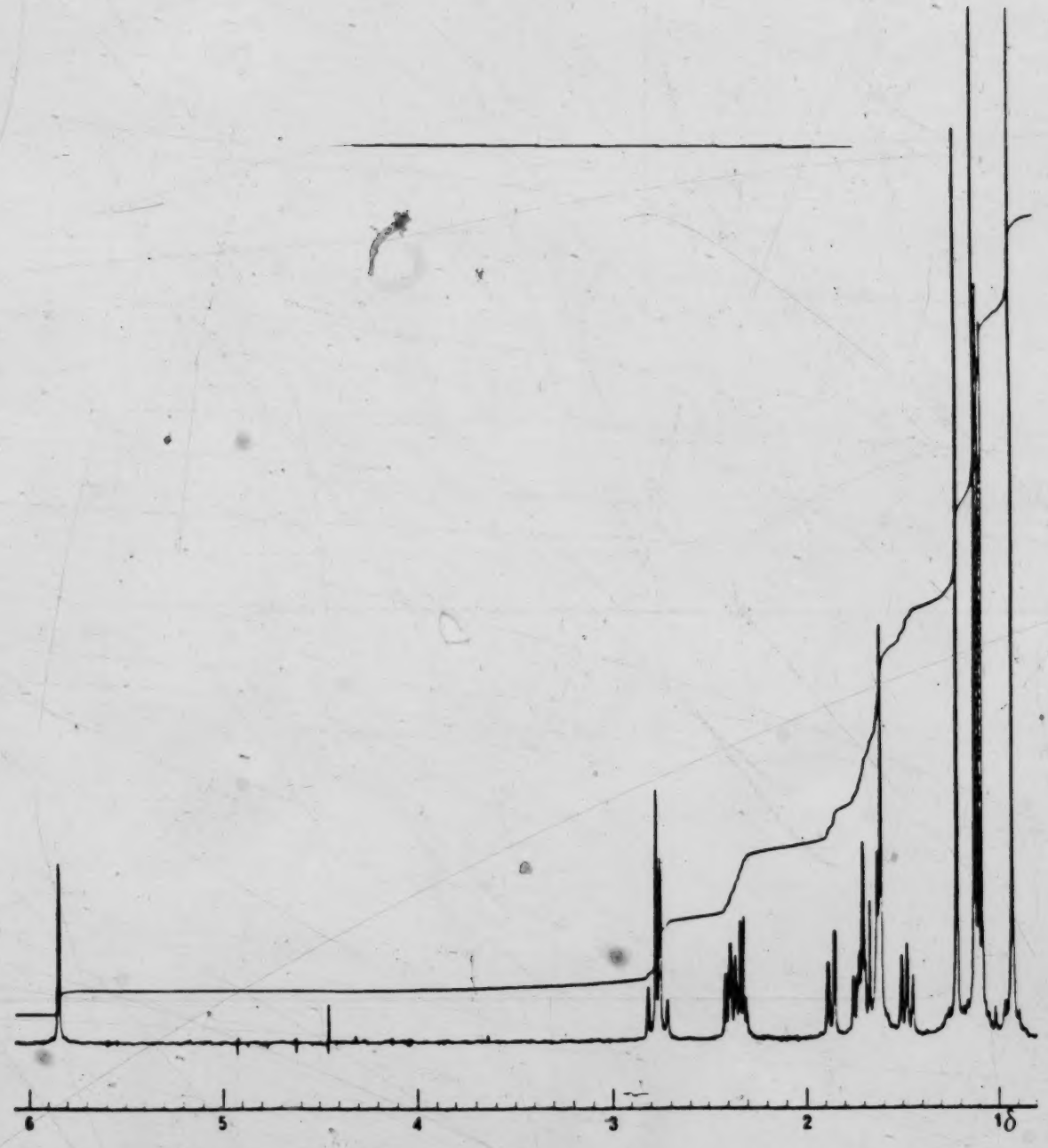

Figure 3. 400 MHz ^{1}H NMR spectrum ($CDCl_3$) of anhydroarthrosporone (21)

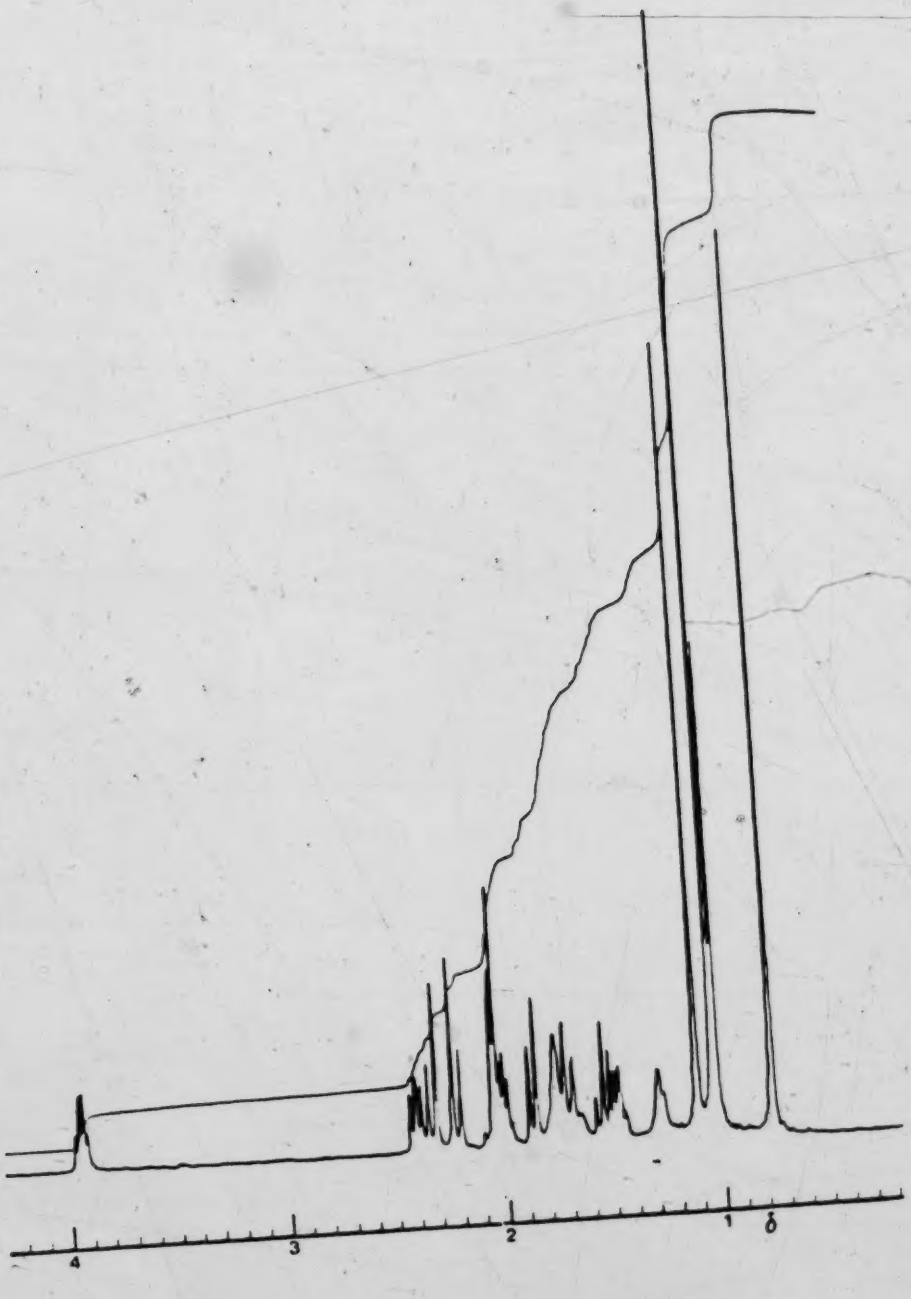

Figure 7. 400 MHz ^1H NMR spectrum ($CDCl_3$) of arthrosporol (22)

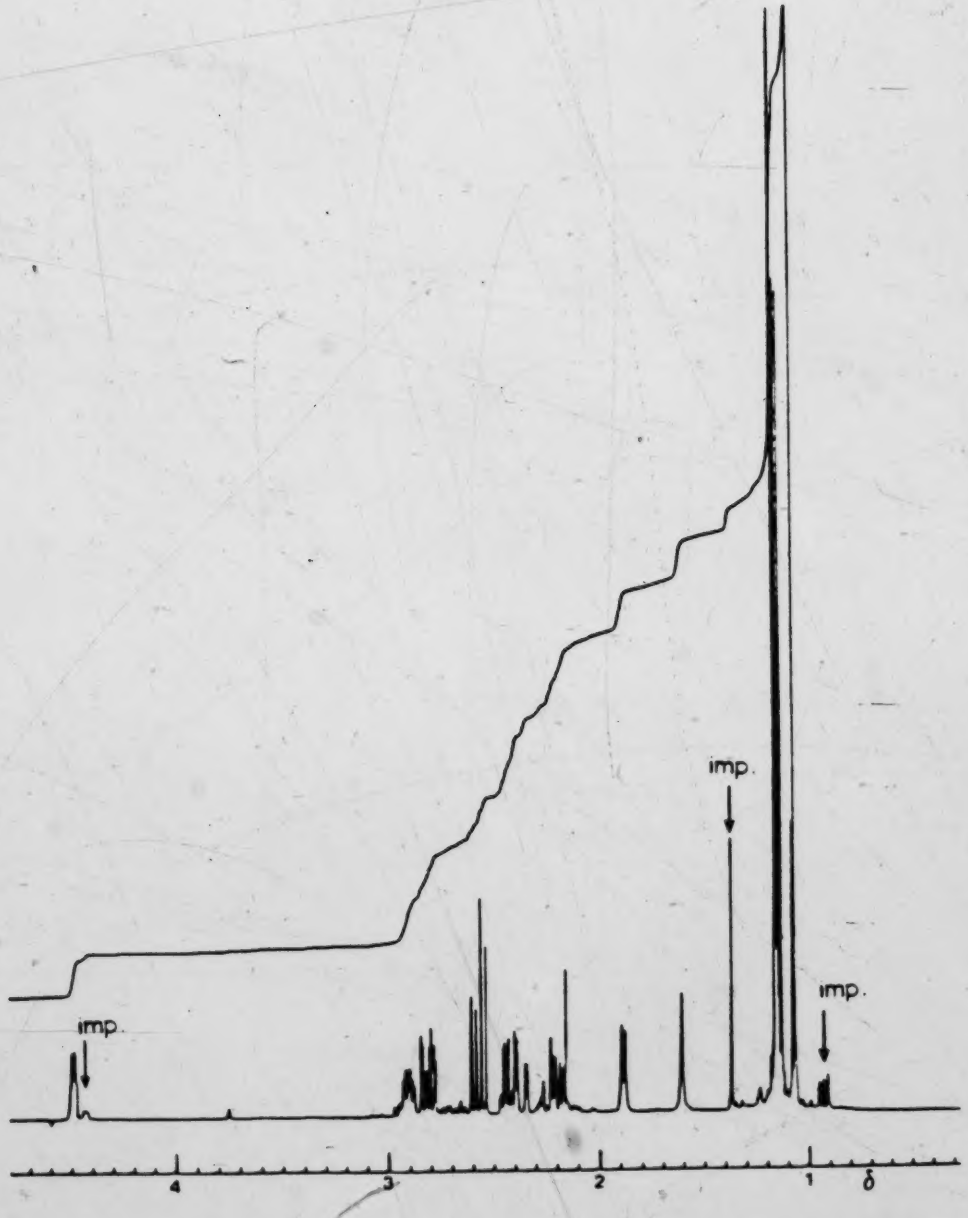

Figure 11. 400 MHz ^1H NMR spectrum ($CDCl_3$) of dehydroarthrosporodione (23)

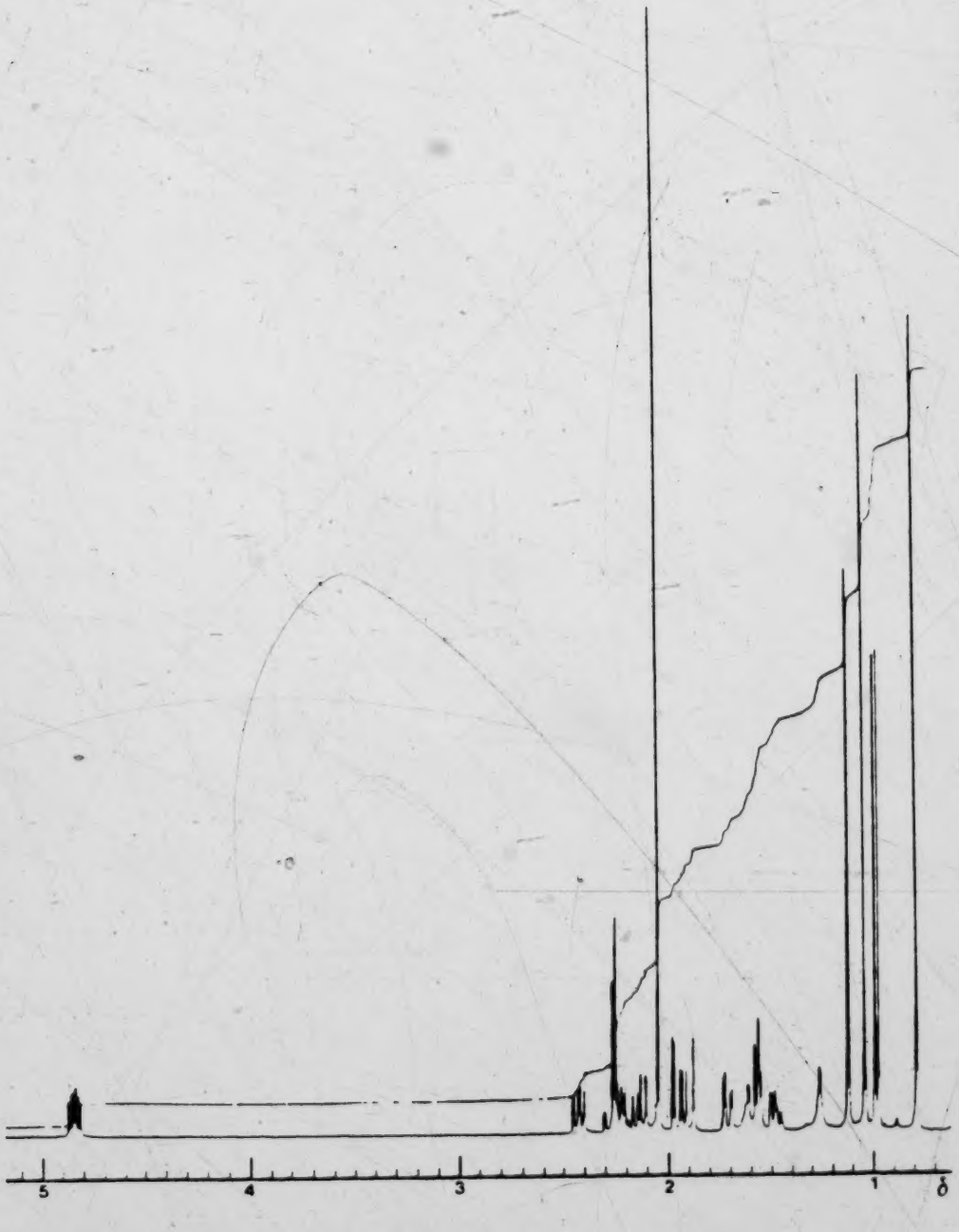

Figure 12. 400 MHz ^{1}H NMR spectrum ($CDCl_3$) of 4-O-acetylarthrosporol (24)

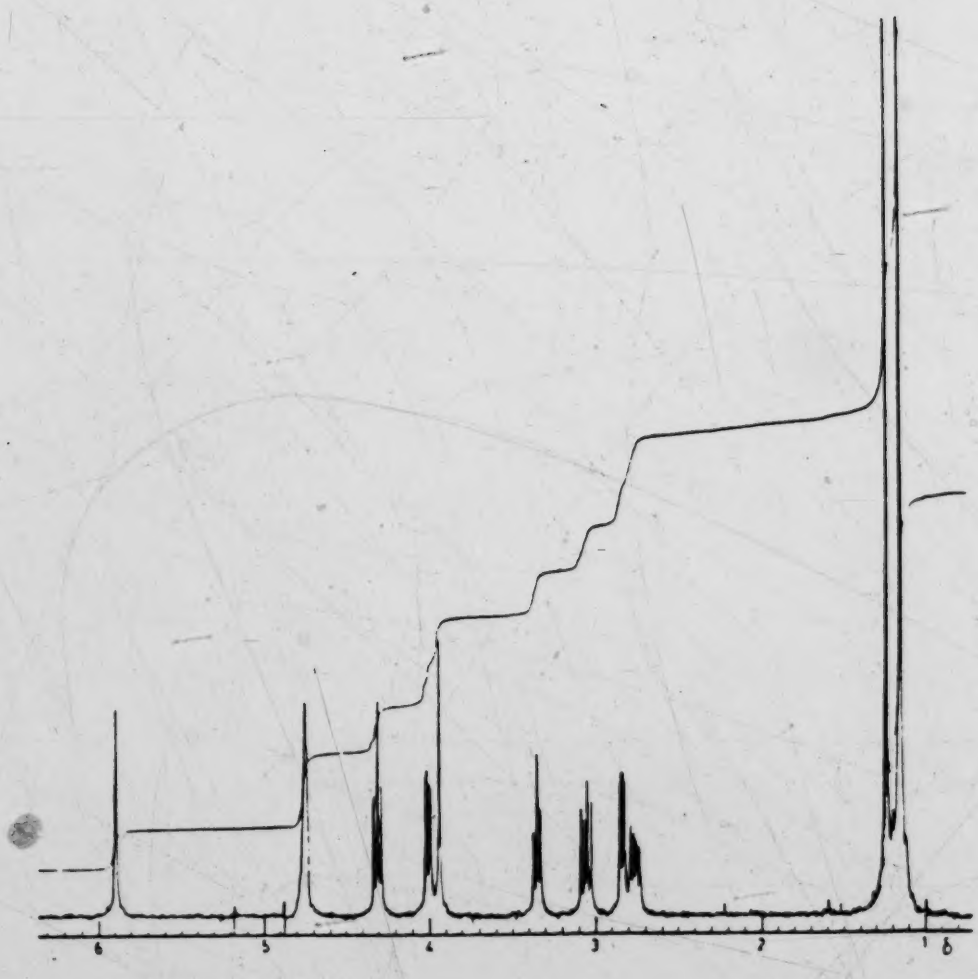

Figure 13. 400 MHz ^{1}H NMR spectrum ($CDCl_3$-D_2O) of tetracyclic ether 25

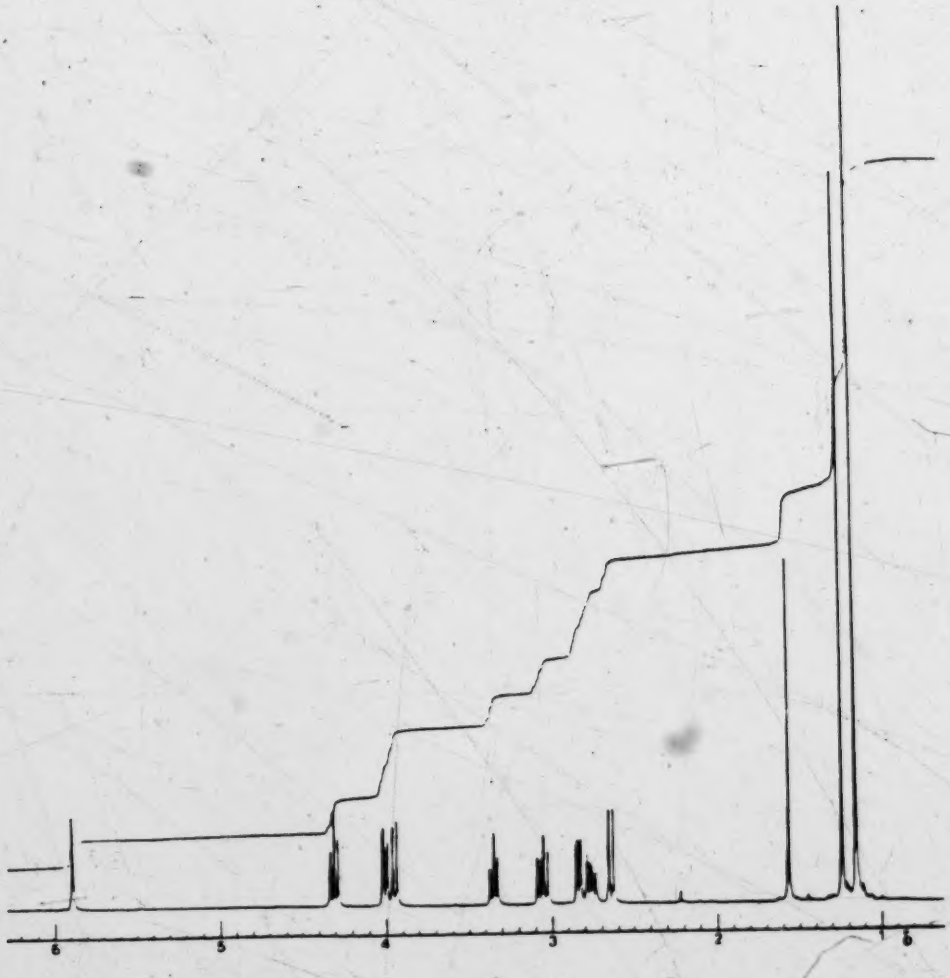

Figure 14. 400 MHz ^{1}H NMR spectrum ($CDCl_3$) of tetracyclic ether 25

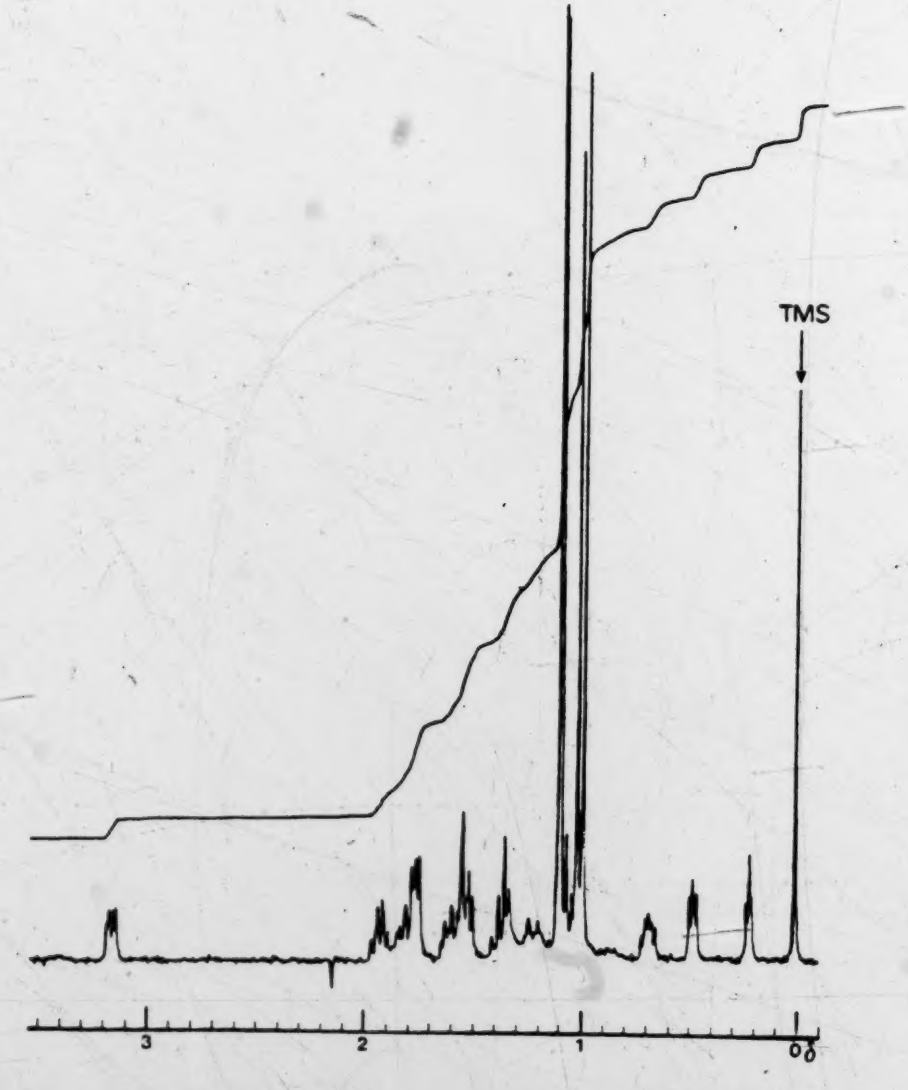

Figure 15. 400 MHz ^{1}H NMR spectrum ($CDCl_3$) of isocyclohumuladiol (26)